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INTERACTIONS BETWEEN CEREAL GRAINS AND OILSEEDS ON MILK FATTY ACID COMPOSITION IN COWS

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INTERACTIONS BETWEEN CEREAL GRAINS AND OILSEEDS ON MILK FATTY ACID COMPOSITION IN DAIRY COWS

Final Report

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A. SUMMARY

The overall objective of this ADF-funded project was to investigate dietary effects on milk fatty acid composition in lactating dairy cows fed supplemental canola and flax. Dietary factors that were investigated include forage particle size and forage:concentrate ratio, the source of ruminally-fermentable non-structural carbohydrate (barley, wheat, oats or corn) and grain processing method (dry rolling versus pelleting). Three major experiments were conducted to investigate the impact of these dietary factors on ruminal fermentation characteristics, nutrient digestion, and milk yield and milk fatty acid composition in lactating dairy cows. In Experiment 1, the aim was to evaluate the effects of length of chop of corn silage and forage:concentrate (F:C) ratio on performance and milk fatty acid profiles in dairy cows supplemented with flaxseed. Our hypothesis was that decreasing forage particle length and F:C ratio would increase unsaturated fatty acid flow to the small intestine and subsequent transfer of these unsaturated fatty acids into milk. Eight Holstein cows (648.1 ± 71.5 kg BW; 109.6 ± 43.6 DIM) were used in a replicated 4 x 4 Latin square design trial with 21-d periods and a 2 x 2 factorial arrangement of dietary treatments. Dietary factors were: 1) F:C ratios (DM basis) of 55:45 and 45:55; and 2) corn silage particle lengths of 9.52 mm and 19.05 mm. All experimental cows received 1 kg of flaxseed to substitute for 1 kg of a rolled barley grain-based concentrate daily. Diets were fed twice daily as a total mixed ration. Corn silage particle length and F:C ratio had no effect on DMI, milk yield and milk composition; however feeding short cut corn silage depressed milk protein yield. Significant particle size x F:C ratio interactions were observed for milk fat proportions of $C_{16:0}$, $C_{18:1}$ *cis*-9 and $C_{18:2}$ *cis*-9, *trans*-11 (a CLA isomer). At short corn silage particle size, decreasing F:C ratio depressed milk fat proportion of $C_{16:0}$. Conversely, feeding short corn silage at high F:C ratio increased the proportion of $C_{18:1}$ *cis*-9 and $C_{18:2}$ *cis*-9, *trans*-11 in milk fat. The milk fat proportion of $C_{18:2}$ *trans*-10, *cis*-12, a CLA isomer that is associated with

milk fat depression, was not affected by dietary treatment. Our results show that corn silage particle length and F:C ratio influence milk FA profiles in dairy cows fed supplemental flaxseed as a source of polyunsaturated fatty acids.

In Experiment 2, eight multiparous Holstein cows (676 ± 57 kg body weight; 121 ± 17 days-in-milk) were used in a replicated 4 x 4 Latin square design trial with 21-d periods to determine the effects of four non-structural carbohydrate (NSC) sources on performance and milk fatty acid profiles in dairy cows. Four cows in one Latin square were fitted with permanent rumen cannulae. The NSC sources evaluated were corn, barley, oats and wheat. All experimental diets contained supplemental canola seed as a source of unsaturated fatty acids. Ruminal fermentation characteristics were largely unaffected by diet; however, ruminal valerate concentrations were higher in cows fed wheat compared to those fed barley, corn or oats. Dietary treatment did not affect total tract digestibility of DM, organic matter and neutral detergent fiber; however, total tract starch digestibility was higher in cows fed oats compared with those fed barley, corn or wheat. Dry matter (DM) intake tended to be lower in cows fed wheat compared to those fed barley, corn or oats; consequently, milk yield for cows fed wheat tended to be lower compared to cows fed barley, corn or oats. Fat content was higher in milk from cows fed corn than in cows fed barley, wheat or oats. Fat yield was higher in cows fed barley, corn, or oats than in cows fed wheat. Feeding oats decreased milk fat proportions of short-chain ($C_{4:0}$ to $C_{12:0}$) and medium-chain ($C_{14:0}$ to $C_{17:0}$) fatty acids (FA), and increased milk fat proportions of $C_{18:1}$ *trans*-fatty acids, total $C_{18:1}$, $C_{18:2}$ *cis*-9, *trans*-11, and total conjugated linoleic acid (CLA) isomers compared to feeding wheat, barley or corn. These changes in milk FA profiles are likely mediated via alterations in ruminal biohydrogenation pathways and, subsequently, changes in de novo mammary FA synthesis and mammary uptake of preformed FA, presumably derived from dietary sources. Results from this study indicate that in lactating dairy cows fed diets containing corn,

wheat, barley or oats with 2.2 to 3.5% of dietary DM as whole canola seed, fatty acid profiles of milk are different.

In Experiment 3, the objective of this study was to determine the effects of methods of barley grain processing and source of supplemental fat on nutrient intake and digestion, patterns of ruminal fermentation, and milk yield and milk fatty acid composition in dairy cows. Eight Holstein cows (656.3 ± 27.7 kg BW; 79.8 ± 12.3 days-in-milk) were used in a replicated 4 x 4 Latin square design with 28-d periods and a 2 x 2 factorial arrangement of dietary treatments. Four cows in one Latin square were fitted with permanent rumen and duodenal cannulae. Experimental diets contained either dry-rolled barley or pelleted barley in combination with either whole canola or whole flaxseed as supplemental fat sources. Dry matter intake and N intake were higher in cows fed dry-rolled barley compared to those fed pelleted barley. Nitrogen retention was not affected by diet, but fecal N excretion was higher in cows fed dry-rolled barley than in those fed pelleted barley. Ruminal pH was lower (P = 0.045) in cows fed pelleted barley compared to those fed dry-rolled barley. Ruminal concentration of acetate was greater (P = 0.001), whereas ruminal concentration of propionate tended to be lower (P = 0.11), in cows fed dry-rolled barley compared to those fed pelleted barley; consequently, the acetate:propionate ratio was higher (P = 0.01) in cows fed dry-rolled barley compared to those fed pelleted barley. Ruminal concentration of total VFA was unaffected (P > 0.05) by diet. Actual and energy corrected milk yield were not affected by diet. Milk fat content and milk fat yield were higher in cows fed dry-rolled barley compared to those fed pelleted barley. Milk fat content of $C_{18:3}$ was higher (P = 0.005) in cows fed canola compared to those fed flax. Milk fat content of $C_{18:3}$ and $C_{18:2}$ *cis*-9, *trans*-11 were higher in cows fed pelleted barley compared to those fed dry-rolled barley with flax as the source of oilseed, but not with canola (interaction, P < 0.01). Milk fat content of saturated fatty acids decreased (P < 0.001) and that of polyunsaturated fatty acids

increased ($P = 0.003$) in cows fed pelleted barley compared to those fed dry-rolled barley. In summary, milk fatty acid profiles were altered by method of grain processing and source of oilseed.

B. PROJECT BACKGORUND

Oilseeds (e.g., canola, flax, sunflower) have become major crops in western Canada. Associated with this, there has been considerable interest in feeding these full-fat oilseeds to dairy cows and feedlot beef cattle. Oilseeds have high contents of both protein and oil (e.g., full-fat canola seed contains approximately 21-22% protein and 41-43% oil; Hickling, 2001), so their inclusion in ruminant diets may provide a high quality protein and also increase the energy density of the diet, particularly for high-producing dairy cows during early lactation.

In addition to increasing the energy density and improving the amino acid composition of ruminant diets, oilseeds have the potential to alter the fatty acid composition of ruminant food products (milk, meat) such that they contain less saturated and more unsaturated fatty acids. This research area has received considerable attention in recent years. Oilseeds are a good source of unsaturated fatty acids. Most of the animal research has been directed at increasing milk content of unsaturated fatty acids such as linoleic acid (C18:2) and conjugated linoleic acid (CLA, C18:2 *cis*-9, *trans*-11). These unsaturated fatty acids have been associated with beneficial effects on human health, such as reducing the risk of coronary heart disease (Massaro et al., 1999), and protecting against cancer (Ip et al., 1994). Therefore, enriching ruminant food products with these unsaturated fatty acids may make them (food products) more appealing to today's health-conscious consumers. Various researchers have reported increases in milk content of these fatty acids when dairy cows are fed full-fat canola seed or canola oil (see Khorasami and Kennelly, 1998; Delbecchi et al., 2001) or flax (Ward et al., 2002; Soita et al., 2003). However, transfer efficiencies of these unsaturated fatty acids from oilseeds to meat or milk have generally been

relatively poor, primarily because their ruminal lipolysis and subsequent biohydrogenation results in major changes of the profile of fatty acids that are available for digestion and absorption at the small intestine. Therefore, research is needed to develop feeding strategies that will protect dietary unsaturated fatty acids from ruminal biohydrogenation, thereby increasing unsaturated fatty acid transfer from oilseeds to meat or milk.

A variety of factors appear to affect the extent of ruminal biohydrogenation of dietary fatty acids, one of the most important factors being ruminal pH. In vitro studies indicated that decreasing ruminal pH from 6.8 to 5.6 inhibited lipolysis and biohydrogenation of dietary unsaturated fatty acids (van Nevel and Demeyer, 1996). This would increase the content of unsaturated FA in ruminal bacteria and the flow of unsaturated fatty acids to the small intestine, where they can be absorbed and made available for incorporation into milk and meat. However, there is no research that has investigated the feeding of diets that alter ruminal pH as a strategy for protecting unsaturated lipid supplements like full-fat canola seed, flax or sunflower against ruminal biohydrogenation. In addition, no research has examined the effects of source of dietary non-structural carbohydrate (NSC) or different grain processing methods on ruminal biohydrogenation of unsaturated fatty acids and, subsequently, effects on unsaturated fatty acid transfer into meat and milk. In western Canada, barley, wheat, oats and corn are commonly used as the cereal base in ruminant diets. Preliminary data from the Crops-Livestock Interface (CLI) project that is funded by the Agricultural Development Fund (see Christensen, 2003) indicated that these cereals differ in their content of NSC. In addition, the rates and extents of ruminal degradation of NSC from these cereals differ, and this would influence ruminal pH which, in turn, would be expected to influence ruminal unsaturated fatty acid biohydrogenation. Furthermore, cereal grain processing methods such as grinding, dry-rolling or steam-flaking, which are commonly used in western Canadian feed mills, will also alter the rates and extents of

ruminal degradation of NSC. Therefore, the objective of this proposed study is to investigate the effects of source of dietary NSC (barley, wheat, oats or corn) and grain processing method (whole, grinding, dry-rolling, steam-flaking) on ruminal biohydrogenation of unsaturated fatty acids from canola, flax and sunflower, and unsaturated fatty acid flow to the small intestine, and milk yield and milk fatty acid composition in lactating dairy cows.

C. MATERIALS AND METHODS

1. EXPERIMENT 1:

1.1. Objectives:

The objective of this study was to evaluate the effects of corn silage particle length and dietary F:C ratio on performance and milk FA profiles in dairy cows supplemented with flaxseed. Our hypothesis was that decreasing corn silage particle length and dietary F:C ratio would depress ruminal pH which, in turn, would impair ruminal FA biohydrogenation, thus potentially increasing unsaturated FA flow to the small intestine and their incorporation into milk fat.

1.2. Cows and Experimental Design:

Eight Holstein cows (648.1 ± 71.5 kg BW; 109.6 ± 43.6 DIM) were used in this study. The experiment was run as a replicated 4 x 4 Latin square design trial with 21-d periods and a 2 x 2 factorial arrangement of dietary treatments. Animals were cared for and handled in accordance with the Canadian Council on Animal Care regulations, and the University of Saskatchewan Animal Care Committee approved their use for this experiment.

1.3. Experimental treatments, feeding management and sample collection:

The four dietary treatments were formulated by combining 2 factors, each with 2 levels. The dietary factors studied were: 1) F:C ratios (DM basis) of 55:45 and 45:55; and 2) corn silage theoretical lengths of cut (TLC) of 9.52 and 19.05 mm. The F:C ratio of the TMR regularly fed in the Dairy Unit at the University of Saskatchewan was 50:50, and the experimental F:C ratios

were chosen to test the effects of a concentrate-rich (F:C = 45:55) or a forage-rich (F:C = 55:45) diet. The range in F:C ratios chosen was not wide so as to encompass typical F:C ratios that are commonly used by dairy producers in the field. The 9.52-mm corn silage TLC was chosen because it is what we commonly use in the Dairy Unit at the University of Saskatchewan, and the 19.05-mm TLC was used to test the effects of increasing TLC of corn silage. The composition and chemical analysis of experimental diets are shown in Table 1. The 19.05-mm TLC of corn silage was achieved with a forage harvester (New Holland, Model 900) set with 26-tooth sprocket and 12 knives, while the 9.52-mm TLC was achieved with 12-tooth sprocket and 12 knives. The chemical composition of the 9.52-mm and 19.05-mm TLC corn silage are presented in Table 2. Each 21-d experimental period consisted of 14 d of dietary adaptation and 7 d for data collection. Experimental diets were fed twice daily at 0800 and 1530 h as TMR for ad libitum intake. At both the morning and afternoon feeding, all cows received 0.5 kg of flaxseed to substitute for 0.5 kg of a rolled barley grain-based concentrate. The inclusion rate of flaxseed was based on a previous study in our laboratory (see Soita et al., 2003) which indicated that this level was efficacious in inducing changes in milk FA profiles. The flaxseed was top-dressed onto the TMR and mixed thoroughly by hand before feeding. Fatty acid composition (g/100 g of FA) of flaxseed was $C_{12:0}$, 0.04; $C_{14:0}$, 0.16; $C_{14:1}$, 0.03; $C_{16:0}$, 6.28; $C_{16:1}$, 0.15; $C_{18:0}$, 3.32; $C_{18:1}$, 16.7; $C_{18:2}$, 15.2; and $C_{18:3}$, 55.3.

During the 7-d data collection period, individual cow feed intake was recorded daily. TMR samples and orts were collected daily, stored at -20°C, and composited per cow for each experimental period. Pooled TMR and orts samples were analyzed for DM by drying in an oven at 60°C for 48 h (AOAC, 1990), CP using the macro-Kjeldahl procedure (AOAC, 1990), ADF (AOAC, 1990), and NDF (Van Soest et al., 1991). Amylase was used for NDF determination. During each data collection period, particle size distributions were determined for corn silage and

all TMR using the Penn State Particle Separator (PSPS) as described by Heinrichs (1996) and Lammers et al. (1996). The PSPS used was equipped with 3 screens (19.0, 8.0 and 1.18 mm) and a bottom pan. Briefly, approximately 150 to 200 g of as-fed corn silage or TMR was placed on the top screen of the PSPS. The PSPS was shaken as described by Heinrichs (1996). Corn silage and TMR fractions retained on each screen and the bottom pan were then weighed to determine particle size distribution. Experimental cows were milked twice daily at 0500 and 1500 h, and milk weights were recorded during the 7-d data collection period. Milk samples were collected daily from morning and afternoon milkings with or without preservative (2-bromo-2-nitropropane-1-2-diol). Milk samples were then pooled daily based on milk yield. Pooled samples with preservative were immediately submitted to the Provincial Milk Testing Laboratory (Saskatchewan Agriculture, Food and Rural Revitalization, Regina, SK) for compositional analysis. Milk samples were analyzed for CP and fat using a near infrared analyzer (Foss System 4000, Foss Electric, Hillerod, Denmark) according to AOAC (1990). Milk samples without preservative were stored at -20°C until further analysis for FA as described below.

1.4. Fatty acid analysis:

Frozen milk samples for FA analysis were thawed in a 38°C water bath and mixed according to AOAC (1990) method 925.21, prior to milk fat extraction. Milk fat extraction was based on the method described by Mir et al. (1999). Briefly, a 8-ml milk sample was transferred into a screw-capped centrifuge tube and 14 ml of hexane/isopropanol (3:2; v/v) was added. Contents were flushed with N_2 and then shaken for 3 min. The mixture was centrifuged at 1750 x *g* for 5 min at 5°C and the upper layer was transferred into another tube and10 ml hexane/isopropanol was added to the lower layer and re-extracted twice. The three extracts were pooled and 7 ml of 0.47 M aqueous sodium sulfate was added and the upper layer (hexane layer) was collected into pre-weighed tubes and evaporated under N_2 in a 40°C water bath. Similarly,

flaxseed fat was extracted from 5 g of a ground sample as described by Folch et al (1957). Plasma fatty acids were determined on samples collected in tubes containing 15% (wt/vol) potassium EDTA. Briefly, 100 µl of plasma was placed in poly-tetra-ethylene screw-capped pyrex tubes and 4 ml of ethyl ether was added. Contents were shaken for 10 min. The extract was washed with 4 ml of saturated sodium chloride and centrifuged at 1750 x *g* for 5 min, and the organic phase transferred to a new tube. Extraction was repeated twice and the solvent was evaporated to dryness under a stream of N_2. The lipid extract was determined gravimetrically.

A known amount of extracted lipid was derivatized using tetramethylguanidine (TMG) and methanol with heneicosanoic acid ($C_{21:0}$) as the internal standard. Briefly, to about 40 mg of extracted lipid in a test tube, 25 µl of 20 mg/ml $C_{21:0}$ was added to each sample, followed by 400 µl of methanol and 100 µl of TMG. The tubes were flushed with N_2 and heated with caps on in boiling water for 10 min. After cooling, 5 ml of saturated NaCl solution was added followed with 2 ml of petroleum ether. The tubes were flushed with N_2 and placed on a tube rocker for 5 min or until there was clear phase separation. The organic phase was transferred to a new test tube and evaporated under N_2. The contents were re-suspended in 5 ml hexane, ready to determine fatty acid profile by gas chromatography.

Fatty acid analysis was carried out in duplicate on a SP-2650, 100m x 0.25 mm ID, 0.2 µm film column (Sigma Aldrich Ont. Canada) installed in an HPGC using a flame ionization detector with capillary injection system at a split ratio of 1:100. The oven temperature was set at 140°C for 5 min, then raised to 240°C at 4.0°C/min, and then held for 60 min. Helium was used as a carrier gas at a flow rate of 1.1 ml/min. Identification of the fatty acids was by comparison to retention times of known standards (Sigma Aldrich Ont. Canada) and amounts present were determined by calculation based on the internal standard.

1.5. Statistical Analysis:

All data on DMI, milk yield and composition, milk fatty acid composition and blood fatty acid profiles were analyzed as a 4 x 4 replicated Latin square with a factorial arrangement of treatments using the Proc Mixed procedure of SAS (SAS User's Guide, 1998). The statistical model included the effects of square, period, corn silage particle length, F:C ratio, and corn silage particle length x F:C ratio. Significant effects were declared at $P < 0.05$, unless otherwise indicated.

2. EXPERIMENT 2:

2.1. Animals and Experimental Design:

Eight multiparous Holstein cows (676 ± 57 kg BW; 121 ± 17 DIM) were used in this study. The experiment was a replicated 4 x 4 Latin square design trial with 21-d periods and four dietary treatments. Four cows in one Latin square were all fitted with permanent ruminal cannulae and were used in a metabolic study to investigate dietary effects on patterns of ruminal fermentation and total tract diet digestibility. Cows were housed in individual tie stalls and had free access to drinking water throughout the trial. Animals were cared for and handled in accordance with the Canadian Council on Animal Care regulations, and the University of Saskatchewan Animal Care Committee approved their use for this experiment (UCACS Protocol No. 20040048).

2.2. Experimental Treatments, Feeding Management and Sample Collection:

The four dietary treatments examined were different cereal grains as the major source of non-structural carbohydrate (**NSC**), with the concentrate portion of the TMR containing approximately 60% barley, corn, wheat or oats. All cereal grains were dry rolled. The ingredient composition and chemical analysis of the experimental concentrate mixtures is shown in Table 1. Barley, corn, wheat and oats were selected because dairy cow diets in western Canada and the

USA typically contain any one or combinations of these cereal grains as the principal source of energy. Additionally, starch content and the rates and extents of ruminal starch degradation differ with the species of cereal grain. Whole canola seed was added to the concentrate as the major source of dietary PUFA, such that experimental TMR would contain 5 to 6% total fat. Canola seed was selected because it is high in linoleic acid ($C_{18:2}$), which is a precursor for ruminal production of C18:2 *cis*-9, *trans*-11 and $C_{18:1}$ *trans*-11. Canola seed is also relatively high in linolenic acid ($C_{18:3}$), which is a precursor for ruminal production of $C_{18:1}$ *trans*-11. In addition, canola seed is readily available in western Canada and the northern USA. The FA composition (g/100 g of FA) of canola seed was $C_{16:0}$, 5.44; $C_{16:1}$, 0.23; $C_{18:0}$, 1.77; $C_{18:1}$, 57.1; $C_{18:2}$, 22.9; and $C_{18:3}$, 11.7. The forage:concentrate ratio of the TMR was 50:50. The forage component of the TMR was a mixture of barley silage and chopped alfalfa hay. During each data collection period, particle size distribution was determined for the forage mixture using the Penn State Particle Separator (PSPS) as described by Heinrichs (1996) and Lammers et al. (1996). The PSPS used was equipped with 3 screens (19.0, 8.0 and 1.18 mm) and a bottom pan. Briefly, approximately 150 to 200 g of the forage mixture was placed on the top screen of the PSPS. The PSPS was shaken as described by Heinrichs (1996). Forage fractions retained on each screen and the bottom pan were then weighed to determine particle size distribution. The ingredient composition and chemical analysis of experimental diets are shown in Table 2.

Each 21-d experimental period consisted of 14 d of dietary adaptation and 7 d (d 15 to 21) for data collection. Experimental diets were fed twice daily at 0800 and 1530 h as TMR for ad libitum intake. During the 7-d data collection period, individual cow feed intake was recorded daily. TMR samples and orts were collected daily, stored at -20°C, and composited per cow for each experimental period. Composite orts samples were weighted based on daily amounts. Pooled TMR and orts samples were analyzed for DM by drying in an oven at 60°C for 48 h

(AOAC, 1990), OM by ashing at 550°C for at least 8 h, CP using the macro-Kjeldahl procedure (AOAC, 1990), starch (AOAC, 1990), ether extract (AOAC, 1990), ADF (AOAC, 1990), and NDF (Van Soest et al., 1991). Amylase and sodium sulfite were used for NDF determination. Experimental cows were milked twice daily at 0500 and 1500 h, and milk weights were recorded during the 7-d data collection period. On d 17, 18 and 19 of each experimental period, milk samples were collected daily from morning and afternoon milkings with or without preservative (2-bromo-2-nitropropane-1-2-diol). Milk samples were then pooled daily based on milk yield. Pooled samples with preservative were immediately submitted to the Provincial Milk Testing Laboratory (Saskatchewan Agriculture, Food and Rural Revitalization, Regina, SK) for compositional analysis. Milk samples were analyzed for CP, lactose and fat using a near infrared analyzer (Foss System 4000, Foss Electric, Hillerod, Denmark) according to AOAC (1990). Milk samples without preservative were stored at -20°C until further analysis for FA. Frozen milk samples for FA analysis were thawed in a 38°C water bath and mixed according to AOAC (1990) method 925.21, prior to milk fat extraction. Total milk fat was extracted with chloroform/methanol/water in the ratio of 1:1:0.9 as described by Bligh and Dyer (1959). The total lipids were methylated using $NaOCH_3$ as catalyst (Kramer et al., 1997; Chouinard et al., 1999) and analyzed directly by gas chromatography. A Hewlett-Packard, model 5890 Series II GC (Palo Alto, CA) was used, equipped with a flame-ionization detector, a 100-m CP Sil 88 column (Chrompack Inc., Middelburg, The Netherlands), an autosampler and a computer software system (Hewlett-Packard ChemStation A.07), and operated in a split-less mode (0.3 s), with inlet and detector temperatures set at 250°C, and a temperature program from 45 to 215°C [45°C (hold 4 min), rate of 13°C/min to 175°C (hold for 27 min), rate of 4°C to 215°C (hold for 35 min)]. The FA methyl esters and the methyl esters of CLA were identified by comparison with standards from Nu Chek Prep (Elysian, MN).

Blood samples were collected at 4 h post-feeding on d 19 and 20 of the experimental period from the coccygeal vein of each animal using 2.5-cm, 20-gauge needles, and collected into 10-ml heparin-containing tubes (Becton Dickinson, Rutherford, NJ). Plasma was prepared immediately after collection by centrifugation of blood at 1,000 x *g* for 15 min and then stored at -20°C until further analysis. Plasma urea-N (**PUN**) was determined colorimetrically using the diacetyl monoxime method (Procedure No. 0580; Stanbio Laboratory, Boerne, TX). Plasma glucose was determined colorimetrically using the glucose oxidase method (Procedure No. 1070; Stanbio Laboratory, Boerne, TX).

2.3. Ruminal Fermentation Characteristics and Total Tract Diet Digestibility:

Ruminal fermentation characteristics and total tract diet digestibility were determined using the ruminally-cannulated cows. On d 18 of each experimental period, samples of ruminal contents were collected at 0 (just before the morning feeding), 2, 4 and 6 h after feeding from the ventral sac of the rumen. Ruminal fluid samples were obtained by straining ruminal contents through four layers of cheesecloth. Ruminal fluid pH was immediately determined using a Model 265A portable pH meter (Orion Research Inc., Beverly, MA). A 5-ml sub-sample of strained ruminal fluid was mixed with chilled 25% meta-phosphoric acid (H_2PO_4) and stored at -20°C for later determination of VFA. Another 5-ml sub-sample was mixed with 1% H_2SO_4 and stored at -20°C for later determination of ammonia-N (NH_3-N). At the end of the trial, frozen ruminal fluid sub-samples were thawed at room temperature and then centrifuged at 20,000 x *g* for 20 min. Ruminal VFA were separated and quantified by gas chromatography (Varian 3700; Varian Specialties Ltd., Brockville, ON) with a 15-m (0.53-mm i.d.) fused silica column (DB-FFAP column; J and W Scientific, Folsom, CA). Ammonia content of ruminal samples was determined using the method described by Weatherburn (1967) modified to use a microtiter plate reader.

Total tract nutrient digestibility was determined by total collection of feces and urine between d 17 and d 21 of each experimental period. Feces were collected into large steel trays which were positioned over the gutter behind each stall. Total daily fecal output for each cow was mixed thoroughly, quantitatively transferred into a pre-weighed plastic container and weighed. A 2.5% sub-sample was taken daily and stored at -20°C. At the end of the trial, frozen fecal sub-samples were thawed, dried at 55°C, and then ground through a 1-mm screen using a Christy-Norris mill (Christy and Norris Ltd., Chelmsford, England). Ground fecal samples were then composited per cow for each experimental period. Composited feces were analyzed for OM, CP, EE, starch, ADF, and NDF using the methods already described for pooled TMR and orts samples. Total urine output was collected using indwelling Bardex Foley bladder catheters (26 Fr, 75 cc ribbed balloon, lubricious-coated; C. R. Bard Inc., Covington, GA). Bladder catheter insertion was conducted as described by Crutchfield (1968). Catheters were inserted at 0900 h on d 16, and were then connected to urine collection tubing at 0900 h on d 17. Urine was collected into 20-L Carboy polyethylene containers.

2.4. Statistical Analysis:

All data on DMI, milk yield and composition, milk fatty acid composition, total tract nutrient digestibility, and plasma metabolites were analyzed as a 4 x 4 replicated Latin square using the Proc Mixed procedure of SAS (SAS User's Guide, 1998). The model used for all dependent variables was: $Y_{ijkl} = \mu + S_i + C_j(S)_i + P_k + SP_{ik} + T_l + ST_{il} + \varepsilon_{ijkl}$, where Y_{ijkl} = the dependent variable, μ = overall mean, S_i = effect of square, $C_j(S)_i$ = effect of cow (within square), P_k = effect of period, SP_{ik} = interaction of square x period, T_l = effect of treatment (diet), ST_{il} = interaction of square x treatment, and ε_{ijkl} = random residual error. For dependent variables that had repeated measurements (ruminal pH, and ruminal VFA and NH_3-N

concentrations), the repeated measures option within the Proc Mixed procedure of SAS (1998) was used. Treatment means were separated into significant main effects by the PDIFF option of SAS. Effects were considered significant at a probability $P < 0.05$, and trends were reported if $0.05 < P \leq 0.10$.

3. EXPERIMENT 3:

3.1. Objectives:

The objective of this experiment was to determine how interactions between method of barley grain processing (dry-rolling vs pelleting) and source of supplemental fat in the diet (whole canola seed vs whole flaxseed) alter ruminal fermentation characteristics, nutrient digestion, and milk yield and milk fatty acid composition in dairy cows.

3.2. Cows and Experimental Design:

Eight Holstein cows (648.1 ± 71.5 kg BW; 109.6 ± 43.6 DIM) were used in this study. The experiment was run as a replicated 4 x 4 Latin square design trial with 28-d periods and a 2 x 2 factorial arrangement of dietary treatments. Four cows in one Latin square were all fitted with permanent ruminal cannulae and were used in a metabolic study to investigate dietary effects on patterns of ruminal fermentation, and total tract diet digestibility and nutrient balances. Animals were cared for and handled in accordance with the Canadian Council on Animal Care regulations, and the University of Saskatchewan Animal Care Committee approved their use for this experiment.

3.3. Experimental Treatments, Feeding Management and Sample Collection and Sample Analyses:

Experimental treatments were combinations of barley grain processing (dry-rolling or pelleting) and two sources of dietary supplemental fat (whole canola seed and whole flaxseed) as follows: a) dry-rolled barley + whole canola seed; b) dry-rolled barley + whole flax seed; c)

pelleted barley + whole canola seed; and d) pelleted barley + whole flaxseed. The diets were formulated to be isonitrogenous and isolipidic. Complete ingredient and chemical composition of experimental diets are presented in Table 1. Dry-rolled barley was prepared by passing whole barley grain through large rollers (23 x 58 cm). For pelleting, whole barley grain was ground through a 6.25 mm screen in a hammer-mill and then pelleted using a California pellet mill. Both dry-rolled and pelleted barley were from the same source. Experimental diets were fed twice daily at 0900 and 1600 h as total mixed rations (TMR) for ad libitum intake. The forage:concentrate ratio of the TMR was 50:50. The forage component of the TMR was a mixture of barley silage and chopped alfalfa hay. Barley silage contained 35.5% DM and its chemical composition (DM basis) was: OM, 90.9%; CP, 11.3%; NDF, 54.7%; ADF, 35.8%; and EE, 3.42%. Whole canola seed or whole flaxseed was added such that experimental TMR contained approximately 5.3% total fat.

Each 28-d experimental period consisted of 14 d of dietary adaptation and 14 d (d 15 to 28) for data collection. Experimental diets were fed twice daily at 0800 and 1530 h as TMR for ad libitum intake. During the 14-d data collection period, individual cow feed intake was recorded daily. TMR samples and orts were collected daily, stored at -20°C, and composited per cow for each experimental period. Pooled TMR and orts samples were analyzed for DM by drying in an oven at 60°C for 48 h (AOAC, 1990), CP using the macro-Kjeldahl procedure (AOAC, 1990), ADF (AOAC, 1990), and NDF (Van Soest et al., 1991). Amylase was used for NDF determination. Experimental cows were milked twice daily at 0500 and 1500 h, and milk weights were recorded during the 14-d data collection period. On d 17, 18 and 19 of each experimental period, milk samples were collected daily from morning and afternoon milkings with or without preservative (2-bromo-2-nitropropane-1-2-diol). Milk samples were then pooled daily based on milk yield. Pooled samples with preservative were immediately submitted to the

Provincial Milk Testing Laboratory (Saskatchewan Agriculture, Food and Rural Revitalization, Regina, SK) for compositional analysis. Milk samples were analyzed for CP, lactose and fat using a near infrared analyzer (Foss System 4000, Foss Electric, Hillerod, Denmark) according to AOAC (1990). Milk samples without preservative were stored at -20°C until further analysis for FA. Frozen milk samples for FA analysis were thawed in a 38°C water bath and mixed according to AOAC (1990) method 925.21, prior to milk fat extraction. Total milk fat was extracted with chloroform/methanol/water in the ratio of 1:1:0.9 as described by Bligh and Dyer (1959). The total lipids were methylated using $NaOCH_3$ as catalyst (Kramer et al., 1997; Chouinard et al., 1999) and analyzed directly by gas chromatography. A Hewlett-Packard, model 5890 Series II GC (Palo Alto, CA) was used, equipped with a flame-ionization detector, a 100-m CP Sil 88 column (Chrompack Inc., Middelburg, The Netherlands), an autosampler and a computer software system (Hewlett-Packard ChemStation A.07), and operated in a split-less mode (0.3 s), with inlet and detector temperatures set at 250°C, and a temperature program from 45 to 215°C [45°C (hold 4 min), rate of 13°C/min to 175°C (hold for 27 min), rate of 4°C to 215°C (hold for 35 min)]. The FA methyl esters and the methyl esters of CLA were identified by comparison with standards from Nu Chek Prep (Elysian, MN).

Total tract nutrient digestibility was determined by total collection of feces and urine between d 15 and d 19 of each experimental period using the 4 cannulated cows in one Latin square. Feces were collected into large steel trays which were positioned over the gutter behind each stall. Total daily fecal output for each cow was mixed thoroughly, quantitatively transferred into a pre-weighed plastic container and weighed. A 2.5% sub-sample was taken daily and stored at -20°C. At the end of the trial, frozen fecal sub-samples were thawed, dried at 55°C, and then ground through a 1-mm screen using a Christy-Norris mill (Christy and Norris Ltd., Chelmsford, England). Ground fecal samples were then composited per cow for each experimental period.

Composited feces were analyzed for CP using the macro-Kjeldahl procedure (AOAC, 1990), ADF (AOAC, 1990), and NDF (Van Soest et al., 1991). Amylase was used for NDF determination. Total urine output was collected using indwelling Bardex Foley bladder catheters (26 Fr, 75 cc ribbed balloon, lubricious-coated; C. R. Bard Inc., Covington, GA). Bladder catheter insertion was conducted as described by Crutchfield (1968). Catheters were inserted at 0900 h on d 16, and were then connected to urine collection tubing at 0900 h on d 17. Urine was collected into 20-L Carboy polyethylene containers.

Ruminal fermentation characteristics and duodenal nutrient flows were determined using the four cannulated cows in one Latin square. Starting on d 23 of each experimental period, ruminal pH was measured continuously for 3 consecutive days using the Lethbridge Research Center Ruminal pH Measurement System (Dascor, Escondido, CA) as described by Penner et al. (2006). On d 27 through d 28 of each experimental period, 1000 mL of ruminal contents were collected by manually taking 250 mL of ruminal contents from the cranial ventral, caudal ventral, central, and cranial dorsal rumen through the cannula just before the morning feeding at 0900 h, and then at 2-hourly intervals over a 24-h feeding cycle. Ruminal fluid samples were obtained by squeezing ruminal contents through four layers of cheesecloth. Two 5-mL aliquots of strained ruminal fluid were mixed with 1 mL of metaphosphoric acid (25% wt/vol) and 1 mL of 1% H_2SO_4 and stored at -20°C for later determination of ruminal VFA and NH_3-N, respectively. Ruminal VFA were separated and quantified by gas chromatography (Varian 3700; Varian Specialties Ltd., Brockville, ON) with a 15-m (0.53-mm i.d.) fused silica column (DB-FFAP column; J and W Scientific, Folsom, CA). Ammonia content of ruminal samples was determined using the method described by Weatherburn (1967) modified to use a microtiter plate reader.

3.4. Statistical Analysis:

All data on DMI, milk yield and composition, milk fatty acid composition, apparent total tract nutrient digestibility, and plasma metabolites were analyzed as a 4 x 4 replicated Latin square using the Proc Mixed procedure of SAS (SAS User's Guide, 1998). The general model used was $Y_{ijk} = \mu + \alpha_i + \beta_j + \pi_k + \varepsilon_{ijk}$, where Y_{ijk} = the dependent variable, μ = overall mean, α_i = effect of cow, β_j = effect of period, π_k = effect of treatment, and ε_{ijk} = random residual error. For dependent variables that had repeated measurements (ruminal fermentation characteristics), the repeated measurement option within the SAS (1990) GLM procedure was used. Effects were considered significant at a probability $P < 0.05$, unless otherwise indicated.

D. RESULTS and DISCUSSION

EXPERIMENT 1:

The nutrient composition of experimental diets is presented in Table 1. Mean diet ADF concentration for the concentrate-rich diet (15.6%) and the forage-rich diet (16.9%) were below NRC (2001) recommendations. This was a function of a lower than anticipated ADF concentration of the corn silage (see Table 2). It is possible that this deficiency in dietary ADF might have reduced chewing and rumination activity, thus potentially inducing a more severe ruminal acidosis than we intended with the reduced forage particle size and a higher dietary concentrate level; however, even though we did not measure ruminal pH in the present study, experimental cows did not show any clinical signs of ruminal acidosis such as loose feces or diarrhea. In addition, experimental periods (21 d) were relatively short and any adverse effects of a dietary ADF deficiency would likely be short-term. Table 3 shows particle size distribution of the short- and long-cut corn silage, and of the experimental diets. As expected, the proportion of as-fed long-cut silage retained on the PSPS 19-mm screen was 19 percentage units higher as

compared with short-cut corn silage. The percentage of long-cut corn silage retained on the PSPS 8-mm screen was 14.5 percentage units lower when compared with short-cut corn silage. As a result, experimental diets containing long-cut corn silage had a higher proportion of as-fed particles being retained on the PSPS 19-mm screen, and a lower proportion of as-fed particles retained on the PSPS 8-mm screen when compared with experimental diets containing short-cut corn silage (Table 3). Differences in the percentage of as-fed particles retained on both the PSPS 1.18-mm screen and bottom pan were small for short- and long-cut corn silage, and for experimental diets (Table 3).

Dry matter intake (DMI) was unaffected ($P > 0.05$) by corn silage particle length (Table 4). These findings are in agreement with several other studies (Clark and Armentano, 1998; Bal et al 2000; Onetti et al., 2003) that previously reported a lack of effect of corn silage particle length on DMI. However, other studies have reported a decrease in DMI of dairy cows when corn silage TLC was increased from 19 to 32 mm (Schwab et al., 2002) and from 30 to 40 mm (Timmermans et al., 2000), an observation likely related to higher rumen retention times with long-cut corn silage (Onetti et al., 2003). Corn silage TLC in our study were 9.52 and 19.05 mm, and were below the TLC range used by Timmermans et al. (2000) and Schwab et al. (2002); therefore, any direct comparisons between our study and these studies might be somewhat misleading. Feeding a higher level of concentrate tended ($P = 0.07$) to increase DMI by 1.6 kg/d (Table 4). Yang et al. (2001) observed that dietary F:C ratios of 35:65 or 55:45 had no effects on DMI in cows fed barley silage-based diets; however, Kalscheur et al. (1997) reported a higher DMI in cows fed 25% forage compared to those fed 60% with corn-silage-based diets. Milk yield, 3.5% FCM yield and milk composition were unaffected ($P > 0.05$) by corn silage particle length and level of concentrate; however, cows fed diets containing long-cut corn silage had a higher ($P = 0.04$) milk protein yield compared with cows fed short-cut corn silage (Table 4). The

reason for this higher milk protein yield is unclear, but we surmise that it was presumably because of a numerically lower milk yield for cows fed short-cut corn silage at 45% concentrate (Table 4). The lack of effect of corn silage TLC on milk yield and milk composition is in agreement with findings reported by others (Bal et al., 2000; Schwab et al., 2002; Onetti et al., 2003). Beauchemin et al. (1994) concluded that effects of particle size on milk fat content were likely to be observed when NDF levels were below minimum requirements recommended by NRC (2001). In our study, dietary NDF contents were higher than 28.5%.

The primary objective of the present study was to examine the effects of corn silage particle length and F:C ratio on FA composition of milk fat. We hypothesized that decreasing corn silage particle length and F:C ratio would depress ruminal pH, primarily as a result of reduced chewing and rumination activity due to smaller forage particle size, and higher ruminal VFA production due to feeding more grain. In turn, depressed ruminal pH would impair ruminal FA biohydrogenation as reported previously (Van Nevel and Demeyer, 1996; Loor et al., 2003), thus potentially increasing unsaturated FA flow to the small intestine and their subsequent incorporation into milk fat. Corn silage particle size and F:C ratio had no effect ($P > 0.05$) on milk fat proportions of C_{10} to $C_{14:1}$, and $C_{16:1}$ (Table 5). In dairy cows, short-chain fatty acids (SCFA) i.e., C_{10} to $C_{14:1}$ are mainly derived from de novo synthesis in the mammary gland (Kennelly and Glimm, 1998); therefore, the lack of dietary treatment effects on milk fat levels of these SCFA is indicative of a similar extent of mammary de novo synthesis.

Corn silage particle length had no effect ($P > 0.05$) on milk fat proportion of $C_{16:0}$; however, there was an interaction ($P < 0.01$) between corn silage particle length and F:C ratio (Table 5). In dairy cows, $C_{16:0}$ in milk fat can be derived from both dietary sources and de novo synthesis in the mammary gland (Grummer, 1991). There was significant interaction ($P < 0.05$) between corn silage particle length and concentrate level on milk fat proportions of $C_{18:1}$ *cis*-9,

$C_{18:1}$ *trans*-10 and $C_{18:2}$ *cis*-9, *trans*-11 (Table 5). Feeding long-cut corn silage at high F:C ratio increased the proportions of $C_{18:1}$ *cis*-9 and $C_{18:2}$ *cis*-9, *trans*-11 in milk fat. Corn silage particle length and dietary F:C ratio had no effect ($P > 0.05$) on milk fat content of $C_{18:2}$ *trans*-10, *cis*-12 (Table 5), also a key CLA isomer. The changes in milk FA profiles observed in this study, especially for $C_{16:0}$, are in accordance with changes that occur during dietary-induced milk fat depression (Bauman and Griinari, 2001). The presence in milk fat of $C_{18:1}$ *trans* FA, which are usually intermediates of ruminal biohydrogenation, has been associated with milk fat depression. Baumgard et al (2002) demonstrated that $C_{18:1}$ *trans*-10 may be a potent inhibitor of milk fat synthesis. In the current study, we did not measure dietary treatment effects on patterns of ruminal fermentation, but we can surmise that our dietary treatments might have only caused subtle changes in ruminal environment and shifts in biohyrogenation pathways, hence milk fat was not significantly depressed. Flaxseed is rich in $C_{18:3}$ and ruminal biohydrogenation of this FA leads to $C_{18:1}$ *trans*-11 that can be absorbed post-ruminally and be uptaken by the mammary gland for use as a substrate for $C_{18:2}$ *cis*-9, *trans*-11 synthesis via delta-9 desaturase activity. We observed that the effects of corn silage particle length on $C_{18:1}$ *trans*-11, a precursor for CLA, showed significant trends when low concentrate, short-cut corn silage diets were fed. Flaxseed also contains a fair proportion of $C_{18:2}$ (15.2 g/g FA). Ruminal biohydrogenation of $C_{18:2}$ leads to $C_{18:2}$ *cis*-9, *trans*-11 CLA accumulation in the rumen, which can be absorbed post-ruminally and be uptaken by the mammary gland and incorporated into milk fat. A further biohydrogenation of $C_{18:2}$ results in $C_{18:1}$ *trans*-11, which is also a precursor for CLA synthesis via delta-9 desaturase activity in the mammary gland (Griinari et al., 2000). Our observations that both $C_{18:1}$ *trans*-10 and $C_{18:1}$ *trans*-11 in milk fat were increased when low concentrate, short-cut corn silage diets were fed was unexpected, and the reasons for this observation are unclear. In the absence of ruminal measurements, we speculate that the severity of ruminal pH depression due to feeding

short-cut corn silage and/or more concentrate was somewhat mild, such that any changes in ruminal FA biohydrogenation due to altered ruminal pH were small. This is supported by our observation that, across dietary treatments, $C_{18:2}$ *cis*-9, *trans*-11 was the predominant CLA isomer in milk fat as compared to $C_{18:2}$ *trans*-10, *cis*-12 (Table 5). At higher ruminal pH (>5.8-6.0), the predominant CLA isomer produced in the rumen is $C_{18:2}$ *cis*-9, *trans*-11 (Bauman and Griinari, 2001). This study demonstrated that when feeding high concentrate diets, short-cut corn silage will depress milk fat content of CLA; however this is reversed when low concentrate diets are fed.

Blood concentrations of $C_{14:0}$, $C_{14:1}$, $C_{16:1}$, $C_{18:0}$, and $C_{18:3}$ were unaffected ($P > 0.05$) by corn silage particle length and level of dietary concentrate (Table 6). There was significant interaction ($P < 0.05$) between corn silage particle length and level of dietary concentrate on blood concentrations of $C_{16:0}$, $C_{18:1}$ *cis*-9, and $C_{18:2}$ *cis*-9, *cis*-12 (Table 6). Feeding long-cut corn silage at high F:C ratio increased the proportions of $C_{16:0}$ and $C_{18:1}$ *cis*-9 in blood, whereas $C_{18:2}$ *cis*-9, *cis*-12 was increased. Not surprisingly, the trends in blood FA profiles as affected by dietary treatment reflect trends observed in milk FA profiles.

Table 1. Ingredient and chemical composition of experimental diets varying in forage particle length and forage:concentrate ratio.

	55% concentrate		45% concentrate	
Diet ingredients, % DM	Short cut[1]	Long cut	Short cut	Long cut
Corn silage	45.4	45.4	55.3	55.3
Barley grain, rolled	31.2	31.2	20.58	20.58
Canola meal	7.01	7.01	7.59	7.59
Soybean meal	7.01	7.01	7.59	7.59
Corn gluten meal	2.76	2.76	2.78	2.78
Canola oil	1.08	1.08	0.89	0.89
WDDG[2]	1.38	1.38	1.39	1.39
Molasses	1.09	1.09	0.89	0.89
Mineral-Vitamin mix[3]	1.52	1.52	1.57	1.57
Salt	0.31	0.31	0.29	0.29
Sodium Bicarbonate	0.31	0.31	0.29	0.29
Dynamate	0.11	0.11	0.11	0.11
Limestone	0.78	0.78	0.71	0.71
Chemical composition				
DM, %	55.4	56.1	51.6	52.1
CP, % of DM	17.4	17.5	17.5	17.9
ADF, % of DM	15.8	15.4	17.2	16.5
NDF, % of DM	28.5	28.7	30.2	29.0
Crude fat, % of DM	4.3	4.5	5.9	5.6

[1] Corn silage particle length: short cut = 9.5-mm theoretical length of cut (TLC); long cut = 19-mm TLC.

[2]Wheat distillers dried grains.

[3]Contained (kg^{-1} premix; DM basis): 330,000 IU of vitamin A, 60,000 IU of vitamin D, 1000 IU of vitamin E, 16% Ca, 8.5% P, 6.3% Na, 4.5% Mg, 2,100 mg Zn, 1,500 mg Mn, 535 mg Cu, 12 mg Se, 45 mg I.

Table 2. Chemical composition of short cut and long cut corn silage.

	Corn Silage	
Item	Short cut[1]	Long cut
DM, %	30.9 (1.1)	31.0 (0.8)
CP, % of DM	9.7 (0.6)	9.7 (0.7)
ADF, % of DM	23.8 (0.6)	24.4 (0.3)
NDF, % of DM	45.8 (3.1)	45.1 (1.8)

[1] Means (SD) for short cut (9.5 mm theoretical length of cut; TLC) and long cut (19 mm TLC).

Table 3. Penn State Particle Separator (PSPS) determinations (% retained, as is basis) of corn silage and experimental diets fed to dairy cows.

	Corn Silage		55% concentrate		45% concentrate	
Screen size	Short cut[1]	Long cut	Short cut	Long cut	Short cut	Long cut
>19 mm	4.24 (0.18)	23.2 (2.4)	2.35 (0.52)	17.7 (2.7)	2.87 (0.50)	17.3 (3.1)
>8 mm	73.0 (2.4)	58.5 (4.8)	61.2 (3.6)	45.2 (1.9)	65.3 (2.6)	42.6 (1.9)
>1.18 mm	21.7 (2.0)	17.5 (1.9)	33.9 (3.5)	35.6 (2.9)	29.6 (2.0)	31.6 (1.4)
Bottom pan	0.92 (0.50)	1.20 (0.41)	2.42 (0.21)	2.63 (0.22)	2.15 (0.77)	2.48 (0.50)

[1] Means (SD) for short cut corn silage (9.5 mm theoretical length of cut; TLC) and long cut (19 mm TLC).

Table 4. DMI, milk yield and milk composition of Holstein cows supplemented with flaxseed as affected by feeding diets varying in corn silage particle length and forage:concentrate ratio.

	55% concentrate		45% concentrate			Contrasts: *P* value[1]		
Item	Short cut[2]	Long cut	Short cut	Long cut	SE	L[2]	C	L x C
DMI, kg/d	26.4	26.1	24.9	24.5	1.02	0.24	0.07	0.19
Milk yield, kg/d	41.4	42.3	38.9	40.8	1.42	0.37	0.39	0.64
3.5% FCM, kg/d	41.3	41.3	39.0	40.7	1.32	0.38	0.16	0.42
Fat, %	3.44	3.30	3.54	3.45	0.13	0.21	0.32	0.61
Fat yield, kg/d	1.42	1.39	1.32	1.39	0.06	0.69	0.38	0.71
Protein, %	3.04	3.07	3.02	3.09	0.14	0.39	0.61	0.64
Protein yield, kg/d	1.25	1.29	1.17	1.25	0.04	0.04	0.42	0.81

[1]L = main effect of corn silage length of cut; C = main effect of forage:concentrate ratio; L x C = interaction; NS = not significant.

[2]Corn silage particle length: short cut = 9.5-mm theoretical length of cut (TLC); long cut = 19-mm TLC.

Table 5. Milk fatty acid composition (expressed as g/100 g of milk fat) of Holstein cows supplemented with flaxseed as affected by feeding diets varying in corn silage particle length and forage:concentrate ratio

	55% concentrate		45% concentrate					
						Contrasts: *P* value[1]		
Fatty acids	Short cut	Long cut	Short cut	Long cut	SE	L[2]	C	L x C
$C_{10:0}$	3.36	3.62	3.92	3.49	0.23	0.72	0.95	0.14
$C_{12:0}$	3.99	4.23	4.02	4.20	0.19	0.29	0.94	0.87
$C_{14:0}$	13.1	13.4	13.2	13.1	0.35	0.68	0.94	0.65
$C_{14:1}$	1.56	1.53	1.53	1.58	0.10	0.92	0.93	0.66
$C_{16:0}$	30.9	27.2	30.8	32.4	0.86	0.05	0.01	0.01
$C_{16:1}$	1.98	1.86	1.91	1.92	0.07	0.55	0.81	0.35
$C_{18:0}$	12.5	12.3	12.1	12.6	0.41	0.75	0.97	0.45
$C_{15:1}$, *c*9	27.9	25.6	27.8	29.3	0.77	0.44	0.03	0.02
$C_{18:1}$, *c*6	0.39	0.36	0.44	0.35	0.06	0.46	0.98	0.85
$C_{18:1}$, *t*10	1.02	1.19	1.24	1.56	0.04	0.04	0.05	0.04
$C_{18:1}$, *t*11	0.46	0.41	0.56	0.61	0.05	0.41	0.04	0.10
$C_{18:2}$, *c*9, *c*12	2.67	2.77	2.69	2.56	0.15	0.91	0.54	0.47
$C_{18:2}$, *t*10, c12	0.047	0.058	0.056	0.047	0.006	0.94	0.94	0.61
$C_{18:2}$, *c*9, *t*11	0.60	0.57	0.61	0.69	0.02	0.37	0.02	0.02
$C_{18:3}$	0.93	1.01	0.99	0.88	0.02	0.89	0.65	0.17

[1]L = main effect of corn silage length of cut; C = main effect of forage:concentrate ratio; L x C = interaction; NS = not significant.

[2]Corn silage particle length: short cut = 9.5-mm theoretical length of cut (TLC); long cut = 19-mm TLC.

Table 6. Plasma fatty acid composition (expressed as g/100 g of fat) of Holstein cows supplemented with flaxseed as affected by feeding diets varying in corn silage particle length and forage:concentrate ratio

	55% concentrate		45% concentrate					
						Contrast: *P* value[1]		
Fatty acids	Short cut[2]	Long cut	Short cut	Long cut	SE	L	C	L x C
$C_{14:0}$	1.23	1.44	1.22	1.11	0.06	0.39	0.23	0.21
$C_{14:1}$	0.45	0.51	0.42	0.46	0.20	0.61	0.39	0.71
$C_{16:0}$	11.2	12.9	9.89	10.4	0.60	0.49	0.01	0.01
$C_{16:1}$	0.44	0.52	0.49	0.56	0.03	0.26	0.61	0.37
$C_{18:0}$	19.3	19.8	18.6	17.1	0.36	0.42	0.28	0.31
$C_{18:1}$, *c*9	9.31	11.7	9.56	10.1	0.28	0.21	0.03	0.02
$C_{18:1}$, *t*11	0.31	0.23	0.48	0.33	0.11	0.33	0.04	0.64
$C_{18:2}$, *c*9, *c*12	44.6	47.8	49.6	47.9	1.05	0.05	0.04	0.04
$C_{18:3}$	6.12	5.39	6.67	6.32	0.07	0.42	0.61	0.67

[1]L = main effect of corn silage length of cut; C = main effect of forage:concentrate ratio; L x C = interaction; NS = not significant.

[2]Corn silage particle length: short cut = 9.5-mm theoretical length of cut (TLC); long cut = 19-mm TLC.

EXPERIMENT 2:

2.1. Diet Characteristics

The ingredient composition and chemical analysis of experimental TMR is presented in Table 8. The mean DM, CP, ADF and NDF contents were 54.6% ± 0.4, 17.4% ± 0.3, 26.7% ± 0.6, and 33.6% ± 1.4, respectively (Table 8). Mean diet ADF and NDF contents slightly exceeded the NRC (2001) minimum recommendations of 17 to 21% and 25 to 33%, respectively, for the maintenance of proper ruminal function. This was related to higher than anticipated ADF and NDF concentrations of the barley silage used in this study. Diets formulated to contain NDF above the minimum NRC (2001) recommendations can limit DM intake; however, in the present study, average DM intake was 25.4 kg/d, or approximately 3.8% of mean BW, and this falls within the expected range for dairy cows producing >35 kg/d of milk (NRC, 2001). Mean dietary fat was 5.5% ± 0.2, and this falls within the desired range of 5 to 6% total dietary fat. Dietary fat levels exceeding 6 to 7% reduce fiber digestion and, consequently, lower DM intake in dairy cows (NRC, 2001), hence it was desired to maintain total dietary fat below 6% of DM. Percentages of the forage mixture retained on the 19.0-, 8.0- and 1.18-mm screens, and the bottom pan were 3.2, 37.5, 48.2 and 11.1%, respectively.

2.2. DM Intake, Milk Yield and Milk Composition, and Plasma Metabolites

DM intake was unaffected ($P > 0.05$) by dietary treatment; however, cows fed the barley-, corn- or oats-based TMR consumed 2.2, 2.0 and 1.2 kg/d more DM, respectively, compared to those fed the wheat-based TMR (Table 9), and these numerical differences tended ($P = 0.09$) to be significant. Actual milk yield was unaffected ($P > 0.05$) by dietary treatment; however, cows fed the barley-, corn- or oats-based TMR produced 3.4, 2.1 and 1.6 kg/d more milk, respectively, compared to those fed the wheat-based TMR, and these numerical differences tended ($P = 0.10$) to be significant (Table 9). The likely mechanism for the increased milk yields observed was that

cows fed the barley-, corn- or oats-based TMR ate more DM. Based on dietary NE_L estimates from NRC (2001) (Table 8), 1.2 to 2.2 kg/d of DMI would correspond with 1.9 to 3.5 Mcal/d of additional NE_L intake. Assuming caloric values of 9.3, 5.9, and 4.0 kcal/g of milk fat, protein, and lactose, respectively (Arieli et al., 2001), then the extra NE_L intake could correspond to an additional 5.5, 4.7 and 2.9 kg/d of milk in cows fed the barley-, corn-, and oats-based TMR, respectively. Cows fed the barley-, corn-, and oats-based TMR produced 3.4, 2.1 and 1.6 kg/d more milk, respectively, compared to cows fed the wheat-based TMR. Cows fed the wheat-based TMR produced less ($P = 0.02$) 3.5% FCM compared to cows fed the barley-, corn- or oats-based TMR (Table 9). Perusal of the literature indicates that there are very limited comparative studies using diets containing corn, wheat, oats or barley in which production responses have been reported, and production responses have been variable. Tommervik and Waldern (1969), using diets containing wheat, barley, oats or corn, reported similar milk yields across dietary treatments. Similarly, grazing cows supplemented with 3 kg/d of wheat, barley, oats or corn yielded similar amounts of milk (Jeffery et al., 1976). Moran (1986) reported higher FCM yields in cows fed oats-based diets compared to those fed wheat- or barley-based diets, although DM intakes were similar for all diets. To date, a preponderance of comparative studies conducted under North American conditions have investigated production responses in trials comparing fewer cereal grain types, most commonly corn and barley. Grings et al. (1992) and Khorasani et al. (1994) did not observe any dietary effects on milk yield, which supports findings of the present study, with diets containing either corn or barley as the principal source of starch. In contrast, others (McCarthy et al., 1989; Casper et al., 1999) reported stimulatory effects on milk yield in cows fed corn-based compared to barley-based diets. Among other things, responses of lactating cows to different cereal grains depend on the level of dietary inclusion, the basal ration,

physical processing of the cereal grains, and the level of dietary intake (Moran, 1986; Khorasani et al., 2001); therefore, direct comparisons among studies are somewhat difficult.

Fat content was higher ($P = 0.01$) in milk from cows fed the corn-based diet than in cows fed the barley-, wheat- or oats-based TMR (Table 9). Fat yield was higher ($P = 0.04$) in cows fed the barley-, corn-, or oats-based TMR than in cows fed the wheat- based TMR (Table 9). In contrast, Tommervik and Waldern (1969) reported similar milk fat contents and fat yields in cows fed wheat, barley, oats or corn as the principal energy concentrates. Similarly, Moran et al. (1986) reported no differences in milk fat contents in cows fed wheat, barley or oats; however, milk fat yields were higher in cows fed oats compared with those fed barley or wheat. Higher fat content in milk from cows fed corn-based diets compared to those fed barley-based diets have been reported previously (DePeters and Taylor, 1985; Weiss et al., 1989; Khorasani et al., 2001). Dietary differences on milk compositional quality are generally reflective of differences in patterns of ruminal fermentation (Moran, 1986) and in the site and extent of cereal grain digestion (Khorasami et al., 2001); however, as discussed elsewhere, even though numerical differences in ruminal VFA concentrations among dietary treatments were large in the present study, dietary impacts on patterns of ruminal fermentation were not significant, probably reflecting inadequate replication with the experimental design used. Numerically higher ruminal concentrations of acetate and propionate, and decreased ruminal acetate:propionate ratio in cows fed the wheat-based diet may have led to the observed lower milk fat contents compared to cows fed the barley-, corn- and oats-based diets. Previous studies have reported milk fat depression with decreased ruminal acetate:propionate ratios (Griinari et al., 1998; Onetti et al., 2002). Milk protein content ($P = 0.10$) and yield ($P = 0.08$), and milk lactose content ($P = 0.24$) were not different among dietary treatments (Table 9).

Plasma concentrations of glucose (P = 0.80) and PUN (P = 0.97) did not respond to dietary treatment (Table 9). In dairy cows, PUN largely arises from ruminal catabolism of dietary protein, with the resultant ruminal NH_3-N that is not captured for microbial protein synthesis being absorbed into portal blood and converted to urea in the liver (NRC, 2001). In the present study, dietary differences in ruminal concentrations of NH_3-N were absent, so the lack of response in PUN is not surprising.

2.3. Milk Fatty Acid Composition

The major objective of the present study was to examine the effects of the dietary inclusion of barley, corn, wheat or oats as the principal sources of starch on the FA composition of milk fat. Results from this study indicate that milk fatty acid composition was altered by dietary treatment (Table 10). Feeding the oats-based TMR reduced ($P < 0.05$) the proportions of individual short-chain ($C_{4:0}$ to $C_{12:0}$), medium-chain ($C_{14:0}$ to $C_{17:0}$), and total short-chain, medium-chain and saturated FA in milk fat compared to feeding the corn-, wheat- or barley-based TMR (Table 10). Feeding the oats-based TMR increased ($P < 0.05$) milk fat proportions of $C_{18:1}$ *trans*-6 to 11, $C_{18:1}$ *trans*-12, $C_{18:1}$ *cis*-9, $C_{18:1}$ *cis*-12 and total $C_{18:1}$ compared to feeding the wheat-, barley- or corn-based TMR (Table 10). Of particular interest, feeding the oats-based TMR increased ($P < 0.05$) milk fat proportions of $C_{18:2}$ *cis*-9, *trans*-11 and total CLA isomers by 29 to 40% and 28 to 36%, respectively, and total long-chain, mono-unsaturated and poly-unsaturated fatty acids compared to feeding the wheat-, barley- or corn-based TMR (Table 10). To my knowledge, there are no published studies comparing the effects on milk FA composition of feeding wheat, corn, barley or oats as carbohydrate sources in dairy cows receiving a rich source of PUFA like canola seed. In a comparative study with barley and corn, Khorasami et al. (2001) did not observe dietary influences on milk FA composition. In other previous studies, feeding high concentrate diets with supplemental vegetable oils that are rich in $C_{18:2}$ resulted in

increased milk fat contents of $C_{18:1}$ *trans*-10 and $C_{18:2}$ *cis*-9, *trans*-11 (Piperova et al., 2000; Peterson et al., 2003), and these observations are consistent with results from the present study. High concentrate diets reduce ruminal pH, thus leading to alterations in ruminal biohydrogenation of dietary PUFA resulting in higher intestinal flows of $C_{18:1}$ *trans*-FA and CLA isomers which are then available for mammary utilization during milk fat synthesis (Loor et al., 2003). Surprisingly, changes in milk fat profiles of some CLA isomers, total CLA and PUFA among dietary treatments in the present study occurred in the absence of any observed dietary effects on ruminal pH. It is plausible that the severity of ruminal pH depression due to the source of ruminally-degradable starch was somewhat mild, but was still adequate to cause perturbations in ruminal biohydrogenation of dietary PUFA. This is supported by the observation that, across dietary treatments, $C_{18:2}$ *cis*-9, *trans*-11 was the predominant CLA isomer in milk fat as compared to $C_{18:2}$ *trans*-10, *cis*-12 (Table 5). At higher ruminal pH (>5.8-6.0), the predominant CLA isomer produced in the rumen is $C_{18:2}$ *cis*-9, *trans*-11 (Bauman and Griinari, 2001).

In dairy cows, both short-chain and, to a smaller extent, medium-chain FA are mainly derived from de novo synthesis in the mammary gland (Kennelly and Glimm, 1998); therefore results from the present study indicate that feeding the oats-based diet inhibited mammary de novo FA synthesis. To verify if mammary de novo FA synthesis was inhibited in cows fed the oats-based diet compared to those fed the barley-, corn-, or wheat-based diets, yields of individual FA were calculated as milk fat yield (g of milk fat/d; Table 9) multiplied by the proportion (g of individual FA/g of total recovered FA) of individual FA (Table 10). Calculated yields of individual FA (data not presented) followed the same pattern as for their concentration in milk fat, thus indicating that mammary de novo FA synthesis was likely inhibited in cows fed the oats-based diet; however, the decreased yields of short-chain and medium-chain FA was associated with increased yields of long-chain monounsaturated and polyunsaturated FA, which

were presumably derived from dietary sources. This redistribution of milk FA might have potential benefits for human health as $C_{14:0}$ to $C_{16:0}$, which are the most abundant saturated FA in milk, have been associated with hypercholesterolemic effects (McGuire et al., 1997). On the other hand, numerous health benefits have been attributed to increased consumption of long-chain monounsaturated and polyunsaturated FA, including potential inhibition of carcinogenesis (Parodi, 1997), and cardio-protective roles (Massaro et al., 1999). These changes in milk fat profiles of short-chain and medium-chain FA observed in the present study are in accordance with changes that normally occur during dietary-induced milk fat depression (Bauman and Griinari, 2001). Feeding the oats-based diet increased milk fat content of $C_{18:1}$ *trans*-FA, and elevated levels of $C_{18:1}$ *trans*-FA in milk have been associated with milk fat depression. Baumgard et al. (2002) demonstrated that $C_{18:1}$ *trans*-10 may be a potent inhibitor of mammary de novo milk fat synthesis; unfortunately, the protocol for FA analysis used in the present study could not separate $C_{18:1}$ *trans*-6 to 11 isomers. However, milk fat content in cows fed the oats-based diet was not depressed in the present study, although milk fat contents of $C_{18:1}$ *trans*-FA were elevated, and the reasons for this observation are unclear.

2.4. Ruminal Fermentation Characteristics and Total Tract Diet Digestibility

Ruminal pH was unaffected ($P = 0.81$) by the source of dietary fermentable carbohydrate (Table 11). Cereal grains differ in their starch contents and rates and extents of ruminal starch fermentation (Huntington, 1997), with oats and wheat exhibiting a faster and more extensive ruminal starch degradation compared to barley or corn; consequently, it was expected dietary differences in ruminal pH. The reasons for a lack of dietary treatment effects on ruminal pH are unclear, but could be related to the high NDF concentrations which exceeded NRC (2001) minimum recommendations across all dietary treatments. Dietary NDF concentration is inversely related to ruminal pH as higher dietary NDF promotes less ruminal acid production, and

stimulates chewing and saliva production (NRC, 2001), which could have resulted in smaller, undetectable differences in ruminal pH across dietary treatments. Additionally, ruminal pH was measured in spot samples obtained via ruminal cannula at various times post-feeding, and this might not necessarily be representative and reflective of treatment differences. In comparative studies with diets containing barley, wheat or oats as the principal starch sources, ruminal pH was unaffected by the choice of cereal grain (Moran, 1986), thereby supporting findings from the present study. Ruminal pH ranged from 5.8 to 6.9 and, as expected, declined with time post-feeding in a similar pattern across dietary treatments (Figure 1).

Ruminal NH_3-N concentration was unaffected ($P = 0.54$) by dietary treatment (Table 11). Moran (1986) fed dairy cows complete diets containing barley, wheat or oats as the principal starch sources and did not observe dietary effects on ruminal NH_3-N concentration. Across dietary treatments, ruminal NH_3-N concentrations ranged from 9 to 24 mg/dL and changes in ruminal NH_3-N concentrations with time post-feeding were similar across diets. Across dietary treatments, mean ruminal NH_3-N concentrations at all sampling times post-feeding were >5 mg/dL, which has been suggested to be optimum for maximum microbial protein synthesis (Satter and Slyter, 1974). Ruminal concentrations of acetate ($P = 0.29$), propionate ($P = 0.42$), butyrate ($P = 0.39$), isobutyrate ($P = 0.38$), isovalerate ($P = 0.81$) and total VFA ($P = 0.29$), and acetate:propionate ratio ($P = 0.54$) were unaffected by dietary treatment; however, ruminal concentration of valerate was higher ($P = 0.05$) in cows fed the wheat-based TMR compared to those fed the barley-, corn- or oats-based TMR (Table 11). As the rate of ruminal starch degradation is different among cereal grains, it is reasonable to expect differences in ruminal VFA patterns when different cereal grains are fed, and it is unclear why dietary differences in ruminal VFA were absent in the present study. It is noteworthy that numerical differences in ruminal acetate, propionate and total VFA concentrations between cows fed the wheat-based diet

compared to those fed the corn-, barley- or oats-based diets were large (Table 11) and a lack of statistical significance probably reflects inadequate replication. These numerically higher ruminal VFA concentrations on the wheat-based diet are consistent with the lower DM intake observed, which would promote a lower ruminal volume and, thereby, higher ruminal VFA concentrations (Bauman et al., 1971). However, in other comparative studies with corn and barley, observed differences in ruminal patterns of VFA have been small or absent (DePeters and Taylor, 1985; Weiss et al., 1989). Reasons for these discrepancies between studies may include differences in feed intake and dietary ingredient composition. Ruminal total VFA concentrations ranged from 82 to 135 mmol/L and, as expected, increased with time post-feeding in a similar pattern across experimental diets.

Dietary treatment did not affect daily faecal output ($P = 0.95$), and total tract digestibility of DM ($P = 0.26$), OM ($P = 0.19$) and NDF ($P = 0.10$) (Table 12). Total tract ether extract digestibility was higher ($P = 0.01$) in cows fed the oats- and corn-based diets compared with those fed the barley- or wheat-based diets (Table 12). Total tract ADF digestibility was higher ($P = 0.05$) in cows fed the corn-based diet compared with those fed the wheat-based diets (Table 12). Total tract NDF digestibility tended to be higher ($P = 0.10$) in cows fed the barley-, corn- or oats-based diet compared with those fed the wheat-based diets (Table 12). Total tract starch digestibility was higher ($P = 0.004$) in cows fed the oats-based diet compared with those fed the barley-, corn- or wheat-based diets (Table 12). Across dietary treatments, total tract starch digestibility was >90%. In dairy cows, the major site of cereal grain starch digestion is the rumen; however, cereal grains differ in their rates and extents of ruminal starch digestion, with oats exhibiting a faster and more extensive ruminal starch degradation compared to wheat, barley and corn (Huntington, 1997). Not surprisingly, a more extensive ruminal degradation for oats is associated with a higher total tract starch digestibility compared to wheat, barley or corn, as

observed in the present study. Moran (1986) also reported a higher total tract starch digestibility in dairy cows fed oats compared to those fed wheat or barley. Cows fed the oats-based diet had a higher ($P = 0.02$) total tract CP digestibility compared to those fed the corn- or wheat-based diets (Table 12). This response might be related to the relative extents of ruminal starch degradation of the cereal grains. Higher levels of undigested starch reaching the hindgut in cows fed the corn- and wheat-based diets compared to those fed the oats-based diet might have promoted more bacterial protein synthesis in the hindgut (Orskov et al., 1970). Because there is no mechanism for hindgut enzymatic digestion of the resultant bacterial protein, it is voided in the feces, thus reducing the total tract CP digestibility (Orskov et al., 1970).

Table 7. Ingredient composition and chemical analysis of grain concentrates fed to lactating dairy cows.

	Experimental diets			
	Barley	Corn	Wheat	Oats
Concentrate ingredients, % DM				
Barley	59.7	-	12.2	-
Corn	-	56.5	-	-
Wheat	-	-	60.0	-
Oats	-	-	-	59.4
Canola seed	10.5	7.7	10.5	6.7
Canola meal	7.4	10.4	3.3	10.5
Soybean meal	11.0	14.0	2.6	12.0
Corn gluten meal	2.0	2.0	2.0	2.0
Wheat distillers dried grains	3.8	3.8	3.8	3.8
Canola oil	0.7	0.7	0.7	0.7
Molasses	1.0	1.0	1.0	1.0
Mineral-vitamin premix[1]	3.0	3.0	3.0	3.0
Salt	0.6	0.6	0.6	0.6
Dynamate	0.3	0.3	0.3	0.3
Chemical composition				
DM, %	89.1	88.2	88.9	90.3
CP, %	18.7	18.7	18.6	18.7
ADF, % of DM	10.5	8.1	8.1	15.3
NDF, % of DM	18.8	13.6	17.1	26.3
Starch, % of DM	34.3	40.9	42.4	26.4
Ether extract, % of DM	7.3	8.1	6.9	8.1

[1]Contained (kg^{-1} premix; DM basis): 330,000 IU of vitamin A, 60,000 IU of vitamin D, 1000 IU of vitamin E, 16% Ca, 8.5% P, 6.3% Na, 4.5% Mg, 2,100 mg Zn, 1,500 mg Mn, 535 mg Cu, 12 mg Se, 45 mg I.

Table 8. Ingredient composition and chemical analysis of experimental TMR fed to lactating dairy cows.

	Experimental diets			
	Barley	Corn	Wheat	Oats
TMR ingredients, % as fed				
Barley silage	54.2	54.2	54.2	54.2
Alfalfa Hay	12.4	12.4	12.4	12.4
Barley	19.9	-	4.06	-
Corn	-	18.8	-	-
Wheat	-	-	19.9	-
Oats	-	-	-	19.8
Canola seed	3.51	2.58	3.51	2.24
Canola meal	2.47	3.47	1.11	3.51
Soybean meal	3.67	4.67	0.87	3.99
Corn gluten meal	0.67	0.67	0.67	0.67
Wheat distillers dried grains	1.27	1.27	1.27	1.27
Canola oil	0.24	0.24	0.24	0.24
Molasses	0.33	0.33	0.33	0.33
Mineral-vitamin premix[1]	0.99	0.99	0.99	0.99
Salt	0.20	0.20	0.20	0.20
Dynamate	0.09	0.09	0.09	0.09
Chemical composition				
DM, %	54.5	54.3	54.3	55.1
CP, % of DM	17.6	17.2	17.4	17.3
ADF, % of DM	26.8	25.9	26.5	27.3
NDF, % of DM	32.7	32.9	33.0	35.7
Starch, % of DM	19.2	21.8	22.4	15.2
Ether extract, % of DM	5.3	5.4	5.4	5.8
Ca, % of DM	0.72	0.68	0.66	0.71
P, % of DM	0.58	0.56	0.58	0.59
NE_L, Mcal/kg of DM[2]	1.61	1.60	1.62	1.59

[1]Contained (kg^{-1} premix; DM basis): 330,000 IU of vitamin A, 60,000 IU of vitamin D, 1000 IU of vitamin E, 16% Ca, 8.5% P, 6.3% Na, 4.5% Mg, 2,100 mg Zn, 1,500 mg Mn, 535 mg Cu, 12 mg Se, 45 mg I.

[2]Estimated from NRC (2001).

Table 9. Dry matter intake, milk yield and milk composition, and plasma metabolites of dairy cows fed diets varying in the source of ruminally-fermentable carbohydrate.

Item	Experimental diets				SEM
	Barley	Corn	Wheat	Oats	
DMI, kg/d	26.2	26.0	24.0	25.2	0.6
Milk yield, kg/d	40.2	38.9	36.8	38.4	0.9
3.5% FCM, kg/d	37.9[a]	38.4[a]	34.8[b]	37.0[a]	0.7
Fat, %	3.20[b]	3.45[a]	3.20[b]	3.34[b]	0.05
Fat yield, kg/d	1.26[a]	1.33[a]	1.16[b]	1.26[a]	0.03
Protein, %	3.00	3.07	3.00	2.96	0.03
Protein yield, kg/d	1.20	1.18	1.10	1.12	0.03
Lactose, %	4.33	4.39	4.35	4.34	0.02
Plasma glucose, mg/dL	46.9	46.9	47.6	46.7	0.9
Plasma urea-N, mg/dL	18.5	18.6	18.9	19.0	0.8

[ab]Means within a row with different superscripts differ ($P < 0.05$).

Table 10. Milk fatty acid composition (expressed as g/100 g of milk fat) of Holstein dairy cows fed diets varying in the source of ruminally-fermentable carbohydrate.

	Experimental diets				
Fatty acids	Barley	Corn	Wheat	Oats	SE
$C_{6:0}$	1.87^{ab}	1.92^{a}	1.80^{ab}	1.75^{b}	0.04
$C_{8:0}$	1.21^{ab}	1.25^{a}	1.17^{ab}	1.10^{b}	0.03
$C_{10:0}$	3.04^{a}	2.94^{a}	2.83^{a}	2.54^{b}	0.07
$C_{11:0}$	0.33^{a}	0.31^{ab}	0.35^{a}	0.26^{b}	0.01
$C_{12:0}$	3.70^{a}	3.51^{a}	3.50^{a}	2.99^{b}	0.11
$C_{14:0}$	12.65^{a}	12.44^{a}	12.54^{a}	10.92^{b}	0.23
$C_{14:1}$, *cis*-9	0.77^{ab}	0.76^{ab}	0.88^{a}	0.66^{b}	0.04
$C_{15:0}$	1.39^{ab}	1.22^{b}	1.63^{a}	1.19^{b}	0.10
$C_{16:0}$	28.90^{b}	29.15^{b}	31.52^{a}	26.28^{c}	0.55
$C_{16:1}$, *cis*-9	1.02^{b}	1.03^{b}	1.28^{a}	1.05^{b}	0.05
$C_{17:0}$	0.52^{ab}	0.47^{b}	0.55^{a}	0.49^{ab}	0.02
$C_{18:0}$	13.08	13.85	12.02	14.67	0.71
$C_{18:1}$, *trans*-4	0.02^{b}	0.02^{b}	0.02^{a}	0.03^{a}	0.002
$C_{18:1}$, *trans*-5	0.01	0.01	0.02	0.02	0.005
$C_{18:1}$, *trans*-6 to 11	2.14^{b}	2.09^{b}	1.85^{b}	2.99^{a}	0.19
$C_{18:1}$, *trans*-12	0.39^{b}	0.39^{b}	0.36^{b}	0.56^{a}	0.03
$C_{18:1}$, *trans*-13 to 14	0.60	0.51	0.57	0.65	0.04
$C_{18:1}$, *trans*-16	0.25^{ab}	0.25^{ab}	0.24^{b}	0.29^{a}	0.01
$C_{18:1}$, *cis*-9	19.97^{b}	20.21^{b}	19.18^{b}	23.05^{a}	0.44
$C_{18:1}$, *cis*-11	1.83	1.64	1.83	1.82	0.07
$C_{18:1}$, *cis*-12	0.47^{b}	0.46^{b}	0.45^{b}	0.62^{a}	0.03
$C_{18:1}$, *cis*-13	0.02	0.02	0.02	0.03	0.002
Total $C_{18:1}$	25.71^{b}	25.60^{b}	24.54^{b}	30.05^{a}	0.55
$C_{18:2}$, *trans*-11, *cis*-15	0.07^{ab}	0.06^{ab}	0.06^{b}	0.08^{a}	0.004
$C_{18:2}$, *cis*-9, *cis*-12	2.00	1.92	1.80	2.06	0.07
$C_{18:2}$ *cis*-9, *trans*-11 CLA[1]	0.50^{b}	0.52^{b}	0.44^{b}	0.73^{a}	0.07
$C_{18:2}$ *trans*-9, *cis*-11 CLA	0.06	0.06	0.06	0.07	0.004

$C_{18:2}$, *trans*-10, *cis*-12 CLA	0.01	0.01	0.01	0.01	0.001
$C_{18:2}$, *trans*-11, *trans*-13 CLA	0.016^{ab}	0.012^{b}	0.016^{ab}	0.023^{a}	0.002
$C_{18:2}$, *trans*-9, *trans*-11 + *trans*-10, *trans*-12 CLA	0.022^{ab}	0.020^{ab}	0.023^{ab}	0.025^{a}	0.001
Total CLA isomers	0.61^{ab}	0.62^{ab}	0.55^{b}	0.86^{a}	0.07
$C_{18:3}$, *cis*-9, *cis*-12, *cis*-15	0.41	0.39	0.35	0.38	0.02
$C_{20:0}$	0.17	0.19	0.16	0.17	0.01
$C_{20:1}$	0.09^{b}	0.08^{b}	0.08^{b}	0.10^{a}	0.003
$C_{20:2n6}$	0.02	0.02	0.02	0.03	0.003
$C_{22:0}$	0.058^{a}	0.058^{a}	0.053^{ab}	0.04^{b}	0.004
$C_{20:4}$ (AA)	0.13	0.12	0.13	0.13	0.01
$C_{20:5n3}$, *(EPA)*	0.04	0.03	0.03	0.03	0.002
Short-chain[2]	10.15^{a}	9.92^{a}	9.65^{a}	8.64^{b}	0.22
Medium-chain[3]	47.10^{ab}	46.78^{b}	50.20^{a}	42.41^{c}	0.81
Long-chain[4]	42.70^{b}	43.24^{b}	40.10^{b}	48.90^{a}	0.94
Monounsaturated[5]	25.81^{b}	25.69^{b}	24.64^{b}	30.16^{a}	0.56
Polyunsaturated[6]	3.53^{ab}	3.40^{ab}	3.19^{b}	3.81^{a}	0.12
Saturated[7]	68.88^{a}	69.14^{a}	70.02^{a}	64.33^{b}	0.63

[ab]Means within a row with different superscripts differ ($P < 0.05$).

[1]CLA = conjugated linoleic acid.

[2]Short-chain = $C_{6:0} + C_{8:0} + C_{10:0} + C_{11:0} + C_{12:0}$.

[3]Medium-chain = $C_{14:0} + C_{14:1} + C_{15:0} + C_{16:0} + C_{16:1} + C_{17:0}$.

[4]Long-chain = $C_{18:0} + C_{18:1} + C_{18:2} + C_{18:3} + C_{20:0}$.

[5]Monounsaturated = $C_{14:1} + C_{16:1} + C_{18:1}$.

[6]Polyunsaturated = $C_{18:2} + C_{18:3}$.

[7]Saturated = $C_{6:0} + C_{8:0} + C_{10:0} + C_{12:0} + C_{14:0} + C_{16:0} + C_{18:0} + C_{20:0}$.

Table 11. Ruminal fermentation characteristics of dairy cows fed diets varying in the source of ruminally-fermentable carbohydrate.

Item	Experimental diets				SEM
	Barley	Corn	Wheat	Oats	
Ruminal pH	6.27	6.18	6.11	6.20	0.11
Ruminal NH_3-N, mg/dL	15.6	16.0	15.6	14.5	1.3
VFA concentrations, m*M*					
Acetate	67.9	71.9	73.1	67.7	2.6
Propionate	21.5	22.8	27.6	22.7	2.7
Butyrate	13.6	14.9	13.7	12.8	0.9
Isobutyrate	1.20	1.31	1.31	1.15	0.08
Valerate	1.87^{b}	1.88^{b}	2.44^{a}	1.73^{b}	0.15
Isovalerate	1.96	1.99	1.93	1.81	0.20
Total VFA	108.3	115.4	120.7	108.7	5.5
Acetate:Propionate ratio	3.23	3.24	2.83	3.12	0.22

[ab]Means within rows with different superscripts differ ($P < 0.05$).

Figure 1. Changes in ruminal pH post-feeding in dairy cows fed diets varying in the source of ruminally-fermentable carbohydrate.

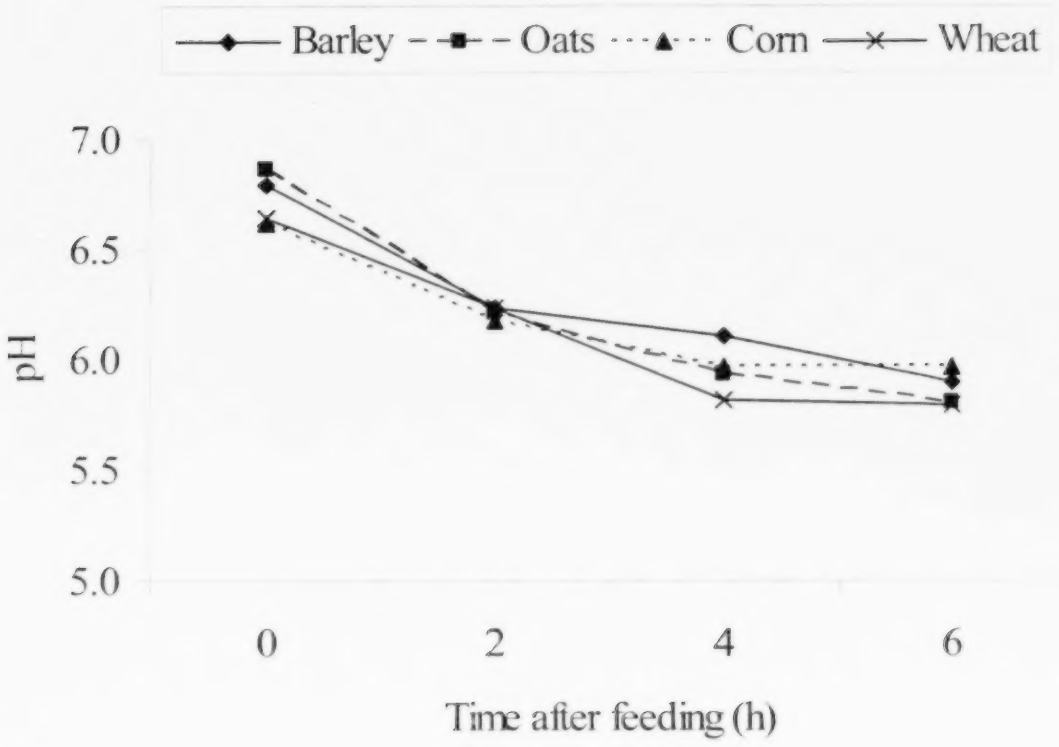
Barley
Oats
Corn
Wheat
7.0
6.5
6.0
5.5
5.0
pH
0
2
4
6
Time after feeding (h)

Table 12. Total tract nutrient digestibility of dairy cows fed diets varying in the source of ruminally-fermentable carbohydrate.

Item	Experimental diets				SEM
	Barley	Corn	Wheat	Oats	
DMI, kg/d	25.8	26.3	24.0	24.8	0.89
Faecal output, kg/d	9.35	9.67	9.73	9.35	0.61
Digestibility, %					
DM	63.7	63.1	59.5	62.1	1.4
OM	65.7	64.6	61.2	63.9	1.3
CP	66.3^{ab}	63.2^{b}	62.9^{b}	70.1^{a}	1.3
Ether extract	57.3^{b}	70.1^{a}	52.3^{b}	74.3^{a}	3.3
ADF	45.2^{ab}	49.2^{a}	37.7^{b}	39.3^{ab}	2.4
NDF	41.1	43.4	30.2	38.5	3.2
Starch	92.6^{b}	91.3^{b}	90.9^{b}	95.8^{a}	0.6

[ab]Means within a row with different superscripts differ ($P < 0.05$).

EXPERIMENT 3:

3.1. Diet Characteristics

The ingredient composition and chemical analysis of experimental TMR is presented in Table 13. The experimental diets were formulated to meet requirements for dairy cows that were 650 kg BW, producing 32 kg/d of milk with 3.6% fat (NRC, 2001). Diets were formulated to be isonitrogenous and isolipidic, and chemical analysis indicates that this was achieved (Table 13). Mean total dietary fat was 5.3%, and this falls within the desired range of <6% total dietary fat. Dietary fat levels exceeding 6 to 7% reduce fiber digestion and, consequently, lower DM intake in dairy cows, hence it was desired to maintain total dietary fat below 6% of DM. Whole canola seed and whole flaxseed were chosen to be used as supplemental fat sources in this experiment for 2 major reasons. Firstly, they are both readily available and are commonly used in dairy cow diets in western Canada and the northern USA. Secondly, canola seed and flaxseed differ in their unsaturated fatty acid contents. The FA composition (g per100 g of FA) of canola seed was: $C_{16:0}$, 5.44; $C_{16:1}$, 0.23; $C_{18:0}$, 1.77; $C_{18:1}$, 57.1; $C_{18:2}$, 22.9; and $C_{18:3}$, 11.7. The fatty acid composition (g/100 g of FA) of flaxseed was: $C_{12:0}$, 0.04; $C_{14:0}$, 0.16; $C_{14:1}$, 0.03; $C_{16:0}$, 6.28; $C_{16:1}$, 0.15; $C_{18:0}$, 3.32; $C_{18:1}$, 16.7; $C_{18:2}$, 15.2; and $C_{18:3}$, 55.3. Therefore, canola seed is high in oleic ($C_{18:1}$) and linoleic acids ($C_{18:2}$), whereas flaxseed is high in linolenic acid ($C_{18:3}$). In addition, these oilseeds differed in their iodine values (IV, calculated from individual fatty acid composition; AOCS, 1989), with mean IV of 112 to 115 and 190 for canola seed and flaxseed, respectively. Because of these differences in the degree of unsaturation of these oilseeds, we expected that their impacts on ruminal microbes and, consequently, on rumen fermentation patterns would be different.

Dietary ruminally-fermentable carbohydrate was altered via barley grain processing (i.e., dry-rolling versus pelleting). Pelleting was expected to decrease particle size, thus shifting the site of carbohydrate digestion from post-ruminal sites to the rumen, which, consequently, would

increase ruminal energy availability. Previous in situ studies in our laboratory clearly indicated a higher soluble starch fraction, a higher degradation rate of the degradable starch fraction, and a higher effective starch degradability of pelleted barley when compared to dry-rolled barley. In the present study, a more acidic ruminal environment in cows fed pelleted barley compared to those fed dry-rolled barley is consistent with a shift in site of starch digestion. Also, barley grain was used because it is the principal cereal grain that is included in dairy cow diets in western Canada, and it is commonly fed either dry-rolled or pelleted.

Patterns of Ruminal Fermentation

Ruminal pH was 0.22 to 0.40 pH units greater ($P = 0.05$) in cows fed dry-rolled barley compared to those fed pelleted barley (Table 14). This was not unexpected as more extensive processing of barley would result in a more rapid and extensive ruminal carbohydrate fermentation (Huntington, 1997), thus increasing the risk of ruminal acidosis. Similarly, Yang et al. (2000) reported a lower ruminal pH in cows fed medium- or flat-rolled barley compared to those fed coarsely-rolled barley. Individual and total concentrations of VFA were greater ($P < 0.05$) in cows fed dry-rolled barley compared to those fed pelleted barley; consequently, the acetate:propionate ratio was higher ($P < 0.001$) in cows fed dry-rolled barley compared to those fed pelleted barley (Table 14). As the rate of ruminal starch degradation is expected to differ with method of grain processing, it is reasonable to expect differences in ruminal VFA patterns, particularly acetate and propionate, when barley grain is fed either dry-rolled or pelleted. There were no time x method of barley processing interactions for the major ruminal VFA; however, there was a time x method of barley processing interaction for valerate ($P = 0.01$) because ruminal concentrations of valerate were similar at feeding, but were higher at all other sampling times in cows fed dry-rolled barley compared to those fed pelleted barley (data not shown). In addition, there was a time x method of barley processing interaction for isovalerate ($P < 0.001$)

because ruminal concentrations of isovalerate were higher at all sampling times during the first 20 h after the first feeding in cows fed dry-rolled barley compared to those fed pelleted barley, but were similar thereafter (data not shown). There were no effects of source of supplemental fat on ruminal VFA (Table 14); however, there was a time x source of supplemental fat interaction ($P < 0.01$) for butyrate because ruminal butyrate concentrations were higher at 8 h after the first feeding, but tended ($P < 0.1$) to be lower at 16 and 20 h after the first feeding, in cows fed whole canola seed compared to those fed whole flaxseed (data not shown). In addition, there was a time x source of supplemental fat interaction ($P = 0.04$) for isobutyrate as ruminal isobutyrate concentrations tended ($P < 0.10$) to be higher at 12 and 20 h after the first feeding, but were higher ($P = 0.01$) at 16 h after the first feeding in cows fed whole canola seed compared to those fed whole flaxseed (data not shown).

Across dietary treatments, ruminal NH_3-N concentrations ranged from 13.9 to 19.9 mg/dL and mean ruminal NH_3-N concentrations at all sampling times post-feeding were >5 mg/dL (data not shown), which has been suggested to be optimum for maximum microbial protein synthesis (Satter and Slyter, 1974). Ruminal NH_3-N concentration was greater ($P = 0.02$) in cows fed dry-rolled barley compared to those fed pelleted barley (Table 14). The lower ruminal NH_3-N concentration in cows fed pelleted barley likely reflects reduced microbial proteolytic activity as a result of a more acidic ruminal environment (Van Soest, 1994). Conversely, a lower ruminal NH_3-N concentration may also be as a result of a higher level of utilization of NH_3-N as a result of more readily available carbohydrate in pelleted barley diets (Hristov et al., 2001). In agreement with our observations, Yang et al. (2000; 2001) also reported a lower ruminal NH_3-N concentration in cows fed more extensively-processed barley compared to those fed coarsely-rolled barley. There was a time x method of barley processing interaction for ruminal NH_3-N concentration ($P < 0.001$) because ruminal NH_3-N concentrations were higher in cows fed dry-

rolled barley compared to those fed pelleted barley for the first 8 h after the first feeding, but ruminal NH_3-N concentrations were similar thereafter (data not shown).

Dry Matter Intake and Total Tract Digestibilities

Dry matter intake (DMI) was affected ($P = 0.04$) by method of barley grain processing, with cows fed dry-rolled barley consuming 1.2 to 3.3 kg/d more DMI compared to those fed pelleted barley (Table 15). This depression in DMI with more extensive barley processing is likely mediated via increased acidity in the rumens of cows fed pelleted barley. The lower DMI in cows consuming pelleted barley compared to those fed dry-rolled barley diets is in agreement with results reported by Yang et al. (2000), who observed a quadratic response in DMI of dairy cows fed barley grain processed to varying degrees, ranging from coarsely-rolled to flat-rolled. In feedlot cattle, Hironaka et al. (1992) observed lower DMI and a higher incidence of digestive disturbances when thinly-rolled or medium-rolled barley diets were fed compared to coarsely-rolled or whole barley diets. In contrast, Yang et al. (2001) reported higher DMI in cows fed flatly-rolled barley compared to those fed coarsely-rolled barley; however, the decrease in ruminal pH (-0.13 pH units) observed with more extensive barley grain processing in that study was not as severe as that observed in the present study (-0.31 pH units), and this could explain the discrepancy in results. Source of dietary supplemental fat did not affect DMI (Table 3). Mean DMI was 22 kg/d, or approximately 3.4% of mean BW, and this falls within the expected range for dairy cows producing 32 kg/d of milk (NRC, 2001). Therefore it is unlikely that feeding supplemental fat decreased DMI. These results are in agreement with those of Ward et al. (2002) in which DMI was similar in cows fed barley-based diets that contained either canola or flax as a supplemental fat source.

Total tract digestibilities of DM, crude fat, ADF, NDF and OM were unaffected ($P > 0.05$) by method of barley processing or source of oilseed (Table 15); however, total tract starch

digestibility was higher ($P < 0.0001$) in cows fed pelleted barley compared to those fed dry-rolled barley (Table 15). In contrast, Yang et al. (2001) reported higher DM and OM digestibilities in cows fed flatly-rolled barley compared to those fed coarsely-rolled barley; however, in agreement with our observations, ADF and NDF digestibilities were not altered. Feeding dry-rolled barley may have reduced the rate of starch fermentability in the rumen while feeding pelleted barley led to a more rapid and complete ruminal starch degradation and, hence, higher starch digestibilities. The lack of effect of barley grain processing on ADF and NDF digestion is somewhat surprising because pelleting barley increased ruminal acidity when compared to dry-rolling. This is possibly due to a higher hindgut fermentation of ADF and NDF in cows fed dry-rolled barley compared to those fed pelleted barley, thus compensating for any depression in ruminal fiber digestion that might have occurred. Across dietary treatments, our estimates of total tract digestibilities of ADF and NDF are similar to previous observations in cows fed similar diets (T. Mutsvangwa, unpublished data); however, it is noteworthy that our estimates of ADF and NDF digestibilities are numerically lower than has been previously observed in cows fed diets with similar ingredient compositions (Yang et al., 2000). Lower ADF and NDF digestibilities observed in the present study might be attributed to depressed ruminal pH. In a previous study (Yang et al., 2000) reporting higher ADF and NDF digestibilities, mean ruminal pH was 0.17 to 0.43 pH units higher when compared to mean ruminal pH values observed in the present study. Across all diets, mean ruminal pH ranged from 5.73 to 6.11 (mean of 5.91), which is lower than the ruminal pH of 6.2 which has been suggested as the minimum ruminal pH for optimum fiber digestion (Van Soest, 1994). Previously, in vitro studies indicated that populations of the principal fiber-digesting bacteria such as *Fibrobacter* spp. and *Ruminococcus albus* decline rapidly when pH falls below 6.0 (Russell and Wilson, 1996). In addition, total dietary fat in the present study was high (>5%; DM basis) and composed mainly of unsaturated fatty acids. In general, supplemental fat sources

that are high in unsaturated fatty acids can have inhibitory effects on ruminal fiber digestion (Schauff et al., 1992; Pantoja et al., 1994), thus partially explaining the lower fiber digestibilities observed in the present study when compared to another study (Yang et al., 2000) in which total dietary fat contents were lower.

Milk Yield and Milk Composition

Actual and energy-corrected milk **(ECM)** were not affected by method of barley grain processing or source of supplemental fat (Table 16). These findings are similar to those by Ward et al. (2002) in which inclusion of flax or canola as supplemental fat source in barley-based diets to attain a dietary fat content of 6% (DM basis) did not affect milk yield. Milk fat content ($P < 0.0001$) and milk fat yield ($P < 0.0001$) were higher in cows fed dry-rolled barley than in cows fed pelleted barley (Table 16). Milk fat contents were 3.47% and 2.67%, and milk fat yields were 1.13 and 0.76 kg/d for cows fed dry-rolled barley and pelleted barley, respectively. Thus, milk fat yield tended ($P = 0.09$) to be lower due to the milk fat depression observed with pelleted barley, and these changes mirrored the numerical changes in milk yield. The milk fat depression observed in cows fed pelleted barley is likely attributed to ruminal fermentation changes, resulting in depressed ruminal pH and associated changes in ruminal biohydrogenation pathways. Previously, in vitro studies indicated that depressed pH altered biohydrogenation pathways of unsaturated fatty acids, such that there was an accumulation of $C_{18:1}$ *trans*-10 and conjugated linoleic acid (CLA) isomers (Van Nevel and Demeyer, 1996; Qui et al., 2004). These unsaturated fatty acids are potent inhibitors of de novo synthesis of short-chain fatty acids in the mammary gland (Bauman and Griinari, 2001). In the present study, post-ruminal flow of CLA isomers and $C_{18:1}$ *trans*-10, or milk fatty acid composition, were not measured. However, the milk fat depression in cows fed pelleted barley suggests that post-ruminal flow of these biohydrogenation intermediates may have been higher than in cows fed dry-rolled barley. Additionally, a lower

ruminal acetate: propionate ratio in cows fed pelleted barley compared to those fed dry-rolled barley may be a possible cause for the observed milk fat depression. Others have reported milk fat depression when ruminal fermentation was altered sufficiently to decrease acetate: propionate ratios (Griinari et al. 1998; Onetti et al. 2002). Milk protein content was higher ($P = 0.005$) in cows fed dry-rolled barley compared to those fed pelleted barley (Table 16). Additionally, feeding whole flaxseed as supplemental fat resulted in higher ($P = 0.01$) milk protein content compared to feeding whole canola (Table 16). Previous studies in which dietary barley grain was processed more extensively (Yang et al., 2001), or whole canola and flaxseed were added as supplemental fat sources (Ward et al., 2002) have also reported changes in milk protein content and milk protein yield.

Results on milk fatty acid composition are presented in Table 17. Milk fat content of $C_{18:3}$ was higher ($P = 0.005$) in cows fed canola compared to those fed flax (Table 17). Milk fat content of $C_{18:3}$ and $C_{18:2}$ *cis*-9, *trans*-11 were higher in cows fed pelleted barley compared to those fed dry-rolled barley with flax as the source of oilseed, but not with canola (interaction, $P < 0.01$) (Table 17). Milk fat content of saturated fatty acids decreased ($P < 0.001$) and that of polyunsaturated fatty acids increased ($P = 0.003$) in cows fed pelleted barley compared to those fed dry-rolled barley (Table 17).

Nitrogen Balance

Cows fed dry-rolled barley consumed 1.2 to 3.3 kg/d more DM compared to those fed pelleted barley, which resulted in cows fed dry-rolled barley consuming 40 to 113 g/d more N compared to those fed pelleted barley ($P = 0.02$; Table 18). However, fecal N excretion was higher ($P = 0.002$) in cows fed dry-rolled barley compared to those fed pelleted barley (Table 5). As a result, cows fed dry-rolled barley had a lower ($P = 0.05$) apparent N digestibility compared to those fed pelleted barley (Table 18). Lower apparent N digestibilities in cows fed dry-rolled

barley compared to those fed pelleted barley suggest that a larger fraction of dietary starch was able to bypass the rumen and was digested in the lower GIT. Shifting starch digestion to the lower GIT promotes hindgut fermentation and leads to more bacterial protein synthesis in the hindgut (Orskov et al., 1970); however, there are no mechanisms for digestion and absorption of the resultant bacterial protein, which is subsequently voided in the feces. Nitrogen use (i.e., N secreted in milk, N retained by the animal, or excreted in urine N) were not affected ($P > 0.05$) by dietary treatment (Table 5). In the present study, we observed a rather large variability in urinary and fecal N excretion, so these data on N balance should be interpreted somewhat cautiously. Spanghero and Kowalski (1997) conducted a meta-analysis of 35 trials that were conducted with dairy cows and found that the mean and median N balance were 39 and 24 g/d, respectively. In the present study, mean N balance ranged from 21.5 to 43.7 g/d, which encompasses the mean N balance reported by Spanghero and Kowalski (1997). Typically, N balance is calculated as N intake-(milk N + fecal N + urine N); therefore, any errors that occur in the measurement of any of the 4 variables that are included in this calculation will be reflected in the N balance data. In addition, N balance data from studies with dairy cows have often been shown to be inherently flawed because the methodologies used often result in unaccountable N losses (Spanghero and Kowalski, 1997). In the present study, it is also possible that urinary and fecal N were underestimated due to possible gaseous N losses particularly during sample collection and processing (e.g., oven drying of fecal samples might have resulted in the loss of volatile N-containing compounds); however, the magnitude of these losses, if any, is unknown. Plasma urea-N concentration was not affected ($P > 0.05$) by dietary treatment (Table 18). In dairy cows, PUN largely arises from ruminal catabolism of dietary protein, with the resultant ruminal NH_3-N that is not captured for microbial protein synthesis being absorbed into portal blood and converted to urea in the liver (NRC, 2001).

Table 13 Ingredient and chemical composition of total mixed rations with dry-rolled or pelleted barley containing canola or flax as supplemental fat sources

	Canola		Flax	
Item	Dry-rolled barley	Pelleted Barley	Dry-rolled barley	Pelleted Barley
Ingredients, % DM				
Barley silage	32.46	32.46	32.48	32.48
Alfalfa hay	17.24	17.24	17.26	17.26
Barley grain	29.81	29.81	29.39	29.39
Canola seed	8.13	8.13	-	-
Flax seed	-	-	8.30	8.30
Soybean meal	3.55	3.55	3.75	3.75
Molasses	0.40	0.40	0.40	0.40
Canola meal	3.49	3.49	3.49	3.49
Corn gluten meal	0.96	0.96	0.96	0.96
Wheat dried distillers grains	1.78	1.78	1.78	1.78
Dynamate[1]	0.18	0.18	0.18	0.18
Salt, Co-I[2]	0.33	0.33	0.33	0.33
Mineral-vitamin supplement[3]	1.66	1.66	1.66	1.66
Chemical composition				
DM, %	57.47	58.31	57.37	58.76
Starch, % of DM	22.96	22.64	24.69	23.73
CP, % of DM	17.42	17.31	17.19	17.00
NDF, % of DM	34.75	35.21	34.77	34.73
ADF, % of DM	21.04	21.37	21.00	20.85
Crude fat, % of DM	5.32	5.33	5.24	5.29

[1]Contained 18% K, 11% Mg, and 22% S.

[2]Salt, cobalt and iodine mix.

[3]Contained (per kg of premix; DM basis): 330,000 IU of vitamin A, 60,000 IU of vitamin D, 1000 IU of vitamin E, 16% Ca, 8.5% P, 6.3% Na, 4.5% Mg, 2,100 mg Zn, 1,500 mg Mn, 535 mg Cu, 12 mg Se, 45 mg I.

Table 14. Ruminal fermentation characteristics in dairy cows fed total mixed rations with dry-rolled or pelleted barley and containing canola or flaxseed as supplemental fat sources

	Canola		Flax			*P* value[1]		
Item	Dry-rolled barley	Pelleted barley	Dry-rolled barley	Pelleted barley	SEM	BP	OS	BP x OS
Ruminal pH	6.13	5.73	5.99	5.77	0.11	0.05	0.66	0.48
Ammonia, mg/dL	19.9	13.9	17.2	15.5	1.3	0.01	0.68	0.13
Volatile fatty acids, m*M*								
Acetate	90.0	74.0	85.8	73.6	3.0	<0.001	0.46	0.54
Propionate	31.1	35.7	28.3	37.3	3.9	0.11	0.88	0.58
Isobutyrate	0.38	0.28	0.34	0.26	0.02	0.002	0.31	0.61
Butyrate	19.0	15.4	17.7	16.1	0.7	0.004	0.73	0.18
Isovalerate	0.96	0.66	1.05	0.63	0.08	<0.001	0.71	0.48
Valerate	1.93	2.58	1.71	2.96	0.43	0.05	0.86	0.50
Total VFA	143.3	128.5	134.9	130.9	7.1	0.21	0.68	0.46
Acetate:propionate ratio	3.0	2.4	3.2	2.2	0.24	0.005	0.98	0.45

[1]BP = barley grain processing (dry-rolled *vs* pelletted); OS = source of supplemental fat in the diet (canola *vs* flax); BP x OS = interaction.

Table 15. Dry matter intakes (DMI) and total tract nutrient digestibilities in dairy cows fed total mixed rations with dry-rolled or pelleted barley and containing canola or flaxseed as supplemental fat sources

	Canola		Flax			*P* value[1]		
Item	Dry-rolled barley	Pelleted barley	Dry-rolled barley	Pelleted barley	SEM	BP	OS	BP x OS
DMI, kg/d	22.3	21.1	23.8	20.5	1.0	0.04	0.65	0.34
Nutrient digestibility, %								
DM	69.2	67.5	68.3	68.4	2.7	0.78	0.99	0.75
Starch	89.8	98.5	89.8	99.0	1.2	<0.0001	0.85	0.85
Crude fat	88.1	88.6	86.7	87.1	1.3	0.8	0.31	0.95
ADF	45.7	37.6	43.3	43.7	4.9	0.44	0.71	0.40
NDF	47.3	42.7	45.8	47.1	4.2	0.71	0.74	0.50
OM	70.6	69.2	69.7	70.3	2.7	0.88	0.97	0.73

[1]BP = barley grain processing (dry-rolled *vs* pelletted); OS = source of supplental fat in the diet (canola *vs* flax); BP x OS = interaction.

Table 16. Milk yield and milk composition in dairy cows fed total mixed rations with dry-rolled or pelleted barley and containing canola or flaxseed as supplemental fat sources

	Canola		Flax			*P* value[1]		
Item	Dry-rolled barley	Pelleted barley	Dry-rolled barley	Pelleted barley	SEM	BP	OS	BP x OS
Actual milk yield, kg/d	32.3	32.4	33.1	28.1	3.42	0.41	0.56	0.39
Energy-corrected milk yield[2], kg/d	31.4	30.1	29.8	27.6	2.02	0.29	0.23	0.74
Fat, %	3.40	2.63	3.54	2.71	0.32	<0.0001	0.37	0.77
Fat yield, kg/d	1.10	0.80	1.15	0.72	0.04	<0.0001	0.78	0.09
Protein, %	2.98	3.18	3.16	3.30	0.15	0.005	0.01	0.62
Protein yield, kg/d	0.96[ab]	1.00[a]	1.03[a]	0.89[b]	0.05	0.13	0.45	0.006

[ab]Means within rows with different superscripts differ ($P < 0.05$)

[1]BP = barley grain processing (dry-rolled *vs* pelletted); OS = source of supplemental fat in the diet (canola *vs* flax); BP x OS = interaction.

[2]Energy-corrected milk yield = milk yield corrected to 3.5% fat and 3.2% protein using the following equation: ECM, kg = (12.82 x kg fat) + (7.13 x kg protein) + (0.323 x kg milk) (Tyrrell and Reid, 1965).

Table 17. Milk fatty acid (FA) composition (expressed as mg FA/100 mg total detected FA) in dairy cows fed total mixed rations with dry-rolled or pelleted barley and containing canola or flaxseed as supplemental fat sources

	Canola		Flax			*P* value[1]		
Fatty acids	Dry-rolled barley	Pelleted barley	Dry-rolled barley	Pelleted barley	SEM	BP	OS	BP x OS
C6:0	1.56	1.43	1.68	1.61	0.05	0.17	0.05	0.66
C7:0	0.02	0.01	0.02	0.03	0.003	0.60	0.34	0.07
C8:0	0.79	0.74	0.91	0.86	0.02	0.11	0.002	0.94
C9:0	0.01	0.02	0.02	0.03	0.002	0.011	0.06	0.99
C10:0	1.54	1.46	1.87	1.74	0.06	0.24	0.003	0.78
C12:0	1.88	1.79	2.26	2.15	0.07	0.29	0.002	0.88
C13:0	0.06	0.06	0.07	0.09	0.01	0.23	0.06	0.22
C14:0	7.56	7.24	8.31	7.98	0.15	0.16	0.006	0.98
C14:1 (trans)	0.40	0.45	0.49	0.54	0.02	0.06	0.004	0.96
C14:1 (cis)	0.68	0.67	0.71	0.87	0.05	0.28	0.12	0.24
C15:0	0.89	0.81	0.94	1.05	0.04	0.79	0.023	0.12
C16:0	20.25	19.88	21.24	20.33	0.40	0.27	0.22	0.64
C16:1 (trans)	0.28	0.26	0.27	0.27	0.02	0.71	0.99	0.76
C16:1 (cis)	1.37	1.60	1.42	1.71	0.03	<0.001	0.11	0.49
C17:0	0.57	0.40	0.26	0.26	0.06	0.32	0.023	0.32
C17:1	0.19	0.22	0.14	0.21	0.02	0.12	0.29	0.65
C18:0	22.57	20.98	21.35	16.62	1.89	0.81	0.07	0.74
C18:1(trans 9 to 11)	5.39	6.66	5.24	7.56	0.41	0.01	0.53	0.38
C18:1(cis-9)	29.44	30.14	26.23	27.02	0.73	0.49	0.01	0.96
C18:2 (t,t)	0.47	0.70	1.23	1.36	0.12	0.32	0.002	0.79

C18:2 (c,c)	1.68	1.72	1.79	2.65	0.25	0.23	0.17	0.27
C18:3 (gamma)	0.25	0.23	0.16	0.16	0.02	0.73	0.01	0.67
C18:3	0.71	0.77	1.47	2.30	0.08	0.002	<0.001	0.005
C20:0	0.11	0.23	0.32	0.10	0.07	0.66	0.69	0.10
C20:1	0.14	0.15	0.06	0.09	0.02	0.44	0.01	0.65
CLA (c9-t11)	0.80	0.94	1.01	1.92	0.08	0.0004	<0.001	0.004
CLA (t10-c12)	0.04	0.10	0.11	0.10	0.038	0.50	0.36	0.35
C20:4	0.12	0.10	0.10	0.12	0.005	0.98	0.77	0.03
C20:5 (EPA)	0.08	0.08	0.11	0.11	0.007	0.88	0.01	0.88
C22:0	0.05	0.05	0.11	0.09	0.011	0.78	0.01	0.50
C22:5 (DPA)	0.08	0.08	0.09	0.09	0.007	0.86	0.41	0.80
C22:6 (DHA)	0.01	0.02	0.03	0.02	0.007	0.76	0.45	0.46
Short-Chain[2]	5.77	5.42	6.72	6.35	0.17	0.16	0.002	0.97
Medium-Chain[3]	32.01	31.31	33.65	33.00	0.49	0.35	0.034	0.97
Long-Chain[4]	61.46	58.88	58.93	59.44	1.53	0.64	0.67	0.49
Monounsaturated[5]	37.57	37.56	34.36	37.97	1.24	0.33	0.44	0.32
Polyunsaturated[6]	3.95	4.29	5.76	8.14	0.26	0.003	<0.001	0.02
Saturated[7]	56.26	52.57	57.94	51.38	0.80	0.001	0.83	0.2315

[1]BP = barley grain processing (dry-rolled *vs* pelletted); OS = source of supplemental fat in the diet (canola *vs* flax); BP x OS = interaction.

[2]Short-chain = $C_{6:0} + C_{8:0} + C_{10:0} + C_{11:0} + C_{12:0}$.

[3]Medium-chain = $C_{14:0} + C_{14:1} + C_{15:0} + C_{16:0} + C_{16:1} + C_{17:0}$.

[4]Long-chain = $C_{18:0} + C_{18:1} + C_{18:2} + C_{18:3} + C_{20:0}$.

[5]Monounsaturated = $C_{14:1} + C_{16:1} + C_{18:1}$.

[6]Polyunsaturated = $C_{18:2} + C_{18:3}$.

[7]Saturated = $C_{6:0} + C_{8:0} + C_{10:0} + C_{12:0} + C_{14:0} + C_{16:0} + C_{18:0} + C_{20:0}$.

Table 18. N balance, N digestibility, and plasma urea-N in dairy cows fed total mixed rations with dry-rolled or pelleted barley and containing canola or flaxseed as supplemental fat sources

	Canola		Flax			*P* value[1]		
Item	Dry-rolled barley	Pelleted barley	Dry-rolled barley	Pelleted barley	SEM	BP	OS	BP x OS
N intake, g/d	622.9	583.1	653.2	539.5	29.1	0.02	0.82	0.23
Fecal N, g/d	159.8	132.4	196.9	116.2	12.8	0.002	0.44	0.06
Urine N, g/d	291.1	261.8	266.5	242.7	54.8	0.64	0.70	0.96
Milk N, g/d	150.5	158.5	162.0	137.0	11.8	0.49	0.68	0.19
N digestibility, %	74.1	77.1	70.5	78.3	2.5	0.05	0.65	0.35
N retention, g/d	21.5	30.4	27.8	43.7	47.9	0.80	0.84	0.94
Plasma urea-N, mg/dL	17.1	14.4	15.3	15.1	1.1	0.20	0.62	0.26

[1]BP = method of barley processing (dry-rolled *vs* pelleted); OS = source of supplemental fat (canola *vs* flax); BP x OS = interaction.

E. CONCLUSIONS and RECOMMENDATIONS

EXPERIMENT 1:

This study demonstrated that the effects of supplementing flaxseed in diets of lactating dairy cows as source of PUFA in milk is influenced by corn silage particle length and F:C ratio. The changes in milk FA profiles observed here are likely due to shifts in ruminal environmental kinetics that, in turn, affect bacterial FA biohydrogenation pathways. An interaction of corn silage particle length and F:C ratio was observed for milk fat contents of $C_{18:1}$ *cis*-9 and $C_{18:2}$ *cis*-9, *trans*-11, as milk fat of cows fed short-cut corn silage at low F:C ratio had higher proportions of these FA. Our results show that corn silage particle length and F:C ratio influence milk FA profiles in dairy cows fed supplemental flaxseed as a source of polyunsaturated fatty acids.

EXPERIMENT 2:

This study demonstrated that the effects of supplementing whole canola seed in diets for lactating dairy cows as a source of PUFA in milk is influenced by the principal source of ruminally-degradable starch. It had been hypothesized that oats > barley = wheat > corn in the extent of ruminal pH depression and, subsequently, increasing the post-ruminal flow of biohydrogenation intermediates via changes in ruminal biohydrogenation pathways. Results indicate that feeding the oats-based diets decreased milk fat content of short- and medium-chain FA, and increased the milk fat content of some CLA isomers, total CLA and PUFA compared to feeding corn-, barley-, or wheat-based diets, which is in support of the hypothesis. These observed changes in milk FA profiles are likely mediated via alterations in ruminal biohydrogenation pathways and, subsequently, changes in de novo mammary FA synthesis and mammary uptake of preformed FA, presumably derived from dietary sources.

EXPERIMENT 3:

In summary, results from this study indicate that method of barley grain processing had a major impact on nutrient intake, total tract nutrient digestion, and milk composition. In addition, milk fat content was higher in cows fed dry-rolled barley compared to those fed pelleted barley, which might not be a desirable outcome within the component-pricing system for milk in Canada. However, method of barley grain processing and source of supplemental fat had major influences on milk fatty acid composition. Milk fat content of $C_{18:3}$ was higher in cows fed canola compared to those fed flax. Milk fat content of $C_{18:3}$ and $C_{18:2}$ *cis*-9, *trans*-11 were higher in cows fed pelleted barley compared to those fed dry-rolled barley with flax as the source of oilseed, but not with canola. Overall, milk fat content of saturated fatty acids decreased and that of polyunsaturated fatty acids increased in cows fed pelleted barley compared to those fed dry-rolled barley, thus making milk more healthful as polyunsaturated fatty acids have been documented to have beneficial health effects in humans.

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I. OTHER

1.1. Peer-Reviewed Manuscripts:

H. W. Soita, M. Fehr, D. A. Christensen, and T. Mutsvangwa. 2005. Effects of corn silage particle length and forage:concentrate ratio on milk fatty acid composition in dairy cows fed supplemental flaxseed. Journal of Dairy Science 88:2813-2819.

1.2. Conference Presentations Made:

H. W. Soita, T. Mutsvangwa, M. Fehr, and D. A. Christensen, 2004. Influence of Corn Silage Particle Length and Forage:Concentrate Ratio on the Impact of Flaxseed Supplementation in Dairy Cow Diets: Effects on Performance and Milk Fatty Acid Composition. Canadian Journal of Animal Science 84:779 (Abstract).

T. Mutsvangwa. 2006. Effects of source of dietary non-structural carbohydrate on milk fatty acid profiles in Holstein dairy cows fed supplemental canola seed. Canadian Journal of Animal Science 86:594 (Abstract).

M. R. Hobin, G. N. Gozho, and T. Mutsvangwa. 2007. Effects of Barley Grain Processing and Source of Oilseed on Milk Fatty Acid Composition in Dairy Cows. Canadian Journal of Animal Science (in press) (Abstract).

1.3. Personnel Involved:

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All staff of the Greenbrae Dairy Research Centre, University of Saskatchewan (involved in animal care)